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THE EVOLUTION OF HUMAN RACES IN THE LIGHT OF THE HORMONE THEORY*

By Prof. Sir Arthur Keith

(Conservator of the Museum and Hunterian Professor, Royal College of Surgeons, London, Eng.)

There is no need for a Herter Lecturer to apologise to Alumni and students of Johns Hopkins University for choosing a subject which has apparently no direct bearing on medical practice. No University has perceived more clearly than Johns Hopkins that rational medical practice must be based on a complete knowledge of the normal machinery of the animal and human body and has, therefore, in the past welcomed every enquiry which was likely to broaden the basis of this knowledge. When we set out to discover how the races of mankind have arisen, we have to make a full demand on all we know concerning the factors which regulate the growth of the human body—particularly on the action of the glands of internal secretion. Much of what the world knows concerning these glands it has learned from Johns Hopkins University—from Howell, Cushing, Goetsch, Abel, Barker, MacCallum and many others. In these lectures I hope to return, with some degree of interest, the preliminary outfit which the anthropologist borrowed from the clinician and pathologist.

* Abstract of Lecture I under the Herter Foundation delivered before the Johns Hopkins University on October 5, 1921.

Darwin, Hunter and the Hormone Theory

I can best introduce the problems to be discussed in these lectures by carrying my audience to Darwin's home, near the village of Down, situated in the chalk uplands to the south of London, where, in the year 1870, the great observer was applying his machinery of evolution to the

"Descent of Man." After he had made use of all the natural agencies at his command, the action of selection, the supposed effects of use and disuse, the manner in which one part of the body is correlated with that of another part, he had to declare that none of these could explain how the Negro had come by one set of characteristic features, the Mongol by another set and the European or Caucasian by a third. The missing agency which Darwin was in search of has been discovered accidentally by medical men working on disorders of nutrition and growth in the out-patient departments and wards of public hospitals. It was working under such circumstances that clinicians came to realize that there is placed in the human, as in the animal body, a group of glands, which, through the formation of chemical substances—or Hormones as Starling named them in 1904—determine the individual and racial characters of every man, woman and child. It is a matter worthy of remark that not only Darwin but also his great predecessor, John Hunter, stood on the verge of this discovery—and yet both were sidetracked. John Hunter successfully transplanted the testes from one fowl to another; he grafted the cock's spur on the hen's leg, and the hen's spur on the cock's leg; his earliest investigation was to show that the human testis which failed to descend was arrested because of imperfect development; no one ever realized more clearly than Hunter did that the growth of all these features of the body, which he named "secondary sexual," depends on the presence of healthy sexual glands. Darwin also realized this to the full, but he concentrated his attention, not on how sexual characters are produced but on how they may be selected and perpetuated once they have come into existence. In the history of discovery we learn as much from the failures of brooding geniuses as from their successes. Both Hunter and Darwin had the misfortune to work ahead of the physiological knowledge of their time.

The Features of Neanderthal Man and of the Acromegalic Contrasted

Soon after 1908, when the Council of the Royal College of Surgeons of England placed me in charge of the Museum which a long line of Conservators had built up round Hunter's glorious nucleus, I had to rearrange the skulls of ancient and modern human races. In one section were two specimens of historical interest; one was a cast of the Neanderthal calvarium which Shaaffhausen had sent to Huxley in 1860 and on which the latter had based his statement that Neanderthal man was the most primitive, most ape-like and most ancient type of humanity known to him. The other specimen was a remarkable modern skull which led Dr. Barnard Davis, the British craniologist, flatly to contradict Huxley by asserting that Neanderthal characteristics could still be found in the population of Europe. I noted with interest that the pituitary fossa in the Barnard Davis skull was double the ordinary size, that all the characteristics of a regulated acromegaly were present and that it was these acromegalic traits which gave the apparent resemblance to the Neanderthal type. At the date of which I write there was ample evidence to prove that a disordered enlargement of the pituitary was directly related to the appearance of acromegalic traits and that the effects could be best accounted for by regarding the pituitary as a centre in which growth-hormones were elaborated. The query naturally arose in my mind, as it did in that of others: Can the pituitary be concerned in the evolution of human races?

At the time I commenced this investigation an important truth was dawning on the minds of European anthropologists. The late Professor Gustav Schwalbe of Strassburg, strongly backed by Dr. Adloff of Berlin, led a movement which completely altered the then prevailing conception of Neanderthal man; Schwalbe held that he must be regarded, not as the Pleistocene ancestor of modern Europeans, but as a separate and extinct species of humanity. With the establishment of Schwalbe's position, we had to alter our mental vista of man's past. All the existing races of mankind are members of a single species but as we go into the far past we find not only separate species of humanity, but separate genera. We have to base our conception of the conditions under which mankind was evolved not on what we see in the modern world of man, but on what we can see now among man's nearest allies—the anthropoid apes. These we find broken up into diverse genera and species, confined to definite localities. All discoveries of recent years justify the belief that in the remote past mankind was represented by numerous local genera and species and that the machinery of evolution must have been both active and effective. The nature of this machinery is not beyond recall; we may safely presume that it may be studied now in living groups of anthropoid apes—amongst gorillas, chimpanzees and orangs. We shall find among anthropoid apes the same hormonic mechanism which shaped the ancient and modern racial types of mankind.

Here, too, before following the straight thread of my lecture, I may mention another line of evidence which has influenced the direction of my investigations. We are apt to think of evolution as a process of the past and that mankind has now come to an evolutionary standstill—so far as racial differentiation is concerned. When we look at the distribution of the chief types of humanity as seen in the modern world it is no longer possible to share in this belief. We cannot explain why the Mongolian type was formerly confined to one region of the earth, the negro to another and the European to a third, or why intermediate types link them together, unless we proceed on the hypothesis that evolution has been and is now at work. In every continent we find human races at all stages of differentiation—from an

incipient state such as is exemplified by the British Anglo-Saxon and Celt, where the degree of physical differentiation is slight, up to a complete stage, such as represented by Negro and white man where the degree of physical differentiation is unmistakable. The quest we set out on is the discovery of the physiological machinery concerned in the differentiation of mankind into its various physical types.

ACROMEGALY IS A TRUE DISORDER OF GROWTH

For the purpose of our search no condition is so instructive as that known to clinicians as Acromegaly. In a short period of years after the onset of this disorder, a new and distinctive type of being is produced which the clinician recognizes at a glance as acromegalic. All the bodily structures and characters involved in the change are just those which the anthropologist knows to be concerned in the differentiation of human races. The texture and frequently the tint of the skin is changed; the growth and distribution of hair alters, the nose, lips, jaws, cranium and thorax undergo characteristic transformations; stature and the proportions of the body and limbs become modified. To study and estimate the degree and nature of these acromegalic changes we must apply the same exact methods as anthropologists employ in the study of human races.

Ten years ago I published the results of a craniological examination of acromegalic skulls [1] and showed the general nature of the changes which had occurred in them. At that time I had not sufficiently appreciated the complexity of the processes involved in the growth of the normal cranium, for, to provide room for the growing brain, new bone has to be laid down at varying rates along certain sutural lines, while along other lines it is being absorbed. No less elaborate is the growth mechanism of the face, for, as the teeth erupt, additional bone is interpolated along certain sutural lines and deposited on certain areas while a process of absorption is taking place along other lines and areas. Each bone of the cranium and face represents a separate growth element and it is only by studying the changes which occur in each element that we come by a precise knowledge of how the face of a boy of five years of age is transformed into the face of an adult man. The result of study along these lines on additional acromegalic material has been to show that the differences between a normal and an acromegalic skull are exactly of the same nature as those seen when we compare the skull of a boy in his thirteenth year with one of an adult man. Beyond any doubt, with the onset of acromegaly a true growth process is reawakened and that reawakening is a continuation of the normal process which should cease when the adult stage is reached. The disorderly enlargement of the pituitary is directly related to the resurrection of the process of growth.

[1] An Inquiry into the Nature of the Skeletal changes in Acromegaly. *Lancet*, April 15, 1911.

ACROMEGALIC CHANGES HAVE A FUNCTIONAL SIGNIFICANCE

It is only when we realize that the size and shape of the skull are determined by the functional purposes which it has to serve and by the manner in which it serves them, that we really profit by craniological enquiry. In the first place the skull has to serve as a brain carrier, providing a cavity with walls so fashioned that additional accommodation can be easily and economically provided for the growing brain. In the second place, the skull has to serve as a carrier of sense organs —the eye, nose and ear. In the third place, it has to provide the whole of the bony scaffolding concerned in mastication, and which also answers, in a secondary degree, to the needs of speech and respiration and deglutition. In the fourth place it has to serve as a complex mobile lever on which the muscles of the neck act.

Let us look for a moment at the alterations undergone by acromegalic skulls in respect to these four functions. Apparently the brain increases in bulk and the cranial cavity in size; at least the mean cranial capacity of the acromegalic skulls at my disposal is distinctly above the average for normal skulls. As a sense carrier there is no change, save as regards the nose. But as regards its third or masticatory function, very remarkable and instructive changes occur. The area on the side of the skull from which the temporal muscle arises, becomes greatly extended, particularly in a forward direction. In the forward extension the frontal bone undergoes a transformation involving changes along the coronal suture, the throwing out of great supra-orbital bulwarks which serve as fulcra of origin for the temporal muscle. The transformation is usually facilitated by an enormous extension of the frontal air sinuses. The origin for the masseter muscle—the zygomatic arch—is strengthened, elongated and lowered in position. The areas and processes from which the pterygoid muscles arise, are also strengthened and extended. The ascending ramus of the mandible, which has to provide an area of insertion for those muscles, elongates greatly and sometimes increases in width as well. All parts of the body of the mandible become the sites of true growth; the alveolar margin, carrying the tooth-sockets, is remodelled and extended. The chin and the lower mandibular border are greatly augmented—strengthening and extending the bony scaffolding on which the floor of the mouth is set. All parts of the upper jaw—with one exception—share in the general enlargement of the masticatory system. The exception is the area carrying the alveolar margin and hard palate; these parts, we shall see anon—may not suffer a perceptible degree of change.

Now the parts just named—the chin, the size and form of the mandible, the prominence of the cheek-bones, the size and projection of the ascending nasal process of the

upper jaw, the degree of development of the supra-orbital ridges—are all of them immediately concerned in giving the face its individual, as well as its racial, characteristics. All of them are linked together to serve in one function—mastication. The pituitary acts not in an anatomical but in a physiological manner; it can somehow stimulate and correlate the growth of all the anatomical elements concerned in a single function. We shall see presently that other hormones, besides those arising in the pituitary, can and do influence the growth of the face. It is sufficient for the meantime to be certain that the characterization of the face—the chief signboard of race—is under the control of a hormone system.

Further evidence in favour of the view that the growth functions of the pituitary are grouped on a physiological —not an anatomical—basis is seen when we study the adaptations undergone by the skull to serve as a lever. To that area of the skull to which the muscles of the neck are attached we may give the name of "nuchal platform"; it is fashioned out of cartilage bone, comprising the whole of the cartilaginous part of the occipital bone and the mastoid part of the temporal bone. In acromegaly the nuchal platform has its area greatly enlarged; all its bounding and intercrossing muscular ridges—comprising the mastoid processes and occipital ridges—are enormously strengthened. The need for some mechanism to correlate the growth of the masticatory bony scaffolding and nuchal platform is made very evident in the skulls of anthropoid apes. The gorilla exerts the strength of its massive body through its huge jaws; hence in this animal a supreme development of the masticatory scaffolding is correlated with an enormous expansion of the nuchal platform. In the chimpanzee, a near relative of the gorilla, the jaws are reduced and so is the nuchal platform. The difference between Neanderthal man and modern man—in respect of jaw and neck—corresponds in degree to that seen between gorilla and chimpanzee. As a boy's jaws grow, his neck enlarges; he continues to take a bigger size in collars as each new tooth erupts. In women the head is poised on a slender neck; their maxillary and nuchal development represent an adolescent stage of the male. In these instances, we see the pituitary acting on a series of anatomical elements moulding and adapting them to a single physiological end. Nuchal characters also serve to differentiate races. It is a remarkable fact that the most extensive facial and nuchal development is found amongst Europeans.

All Systems of the Body Are Affected in Acromegaly

Too much stress cannot be laid on the fact that in acromegaly all the tissue systems of the body are involved. In every case of acromegaly which has received a complete examination, it has been found that not only are the skeletal and cutaneous tissues affected but so too are the circulatory, the pulmonary, the alimentary, renal and connective tissue systems. All of them are involved in overgrowth with the exceptions of the sexual system, which undergoes atrophy, and the nervous system, regarding which our evidence is incomplete. Nor can too much emphasis be laid on another fact that in the later stages of the disease—all of these systems suffer degeneration and atrophy. Some years ago the Museum of the Royal College of Surgeons obtained a subject of this disease —a man who had begun to show symptoms of acromegaly at the age of twenty-four and had died twenty-five years after the onset, the disease having been steadily progressive. Preparations, naked-eye and microscopic, were made from all the systems and organs of this individual and are now preserved in the Museum of the College. Every cell of the body of this subject was found to have suffered in the general disorder of growth. For example, the muscles of mastication had not merely undergone a normal degree of hypertrophy; they had undergone a real process of growth—the individual fibres having increased in length, diameter and number. This was also true of all the muscles of the tongue and mouth. The temporal and facial arteries were abnormally large and their muscular coats greatly increased, but, as was the case throughout the whole arterial system, the inner coat was thickened, it encroached on the arterial lumen. From these facts one infers that the pituitary acts upon all the structural elements which are comprised within a functional unit and somehow keeps their growth in unison.

The Nature of the Disorder Known as Acromegaly

So far I have merely put forward the evidence in support of the view that in Acromegaly we have exposed for us part of the growth-machinery concerned in the differentiation of racial characteristics. The pituitary and substances formed within it are essential parts of the machinery. Before we proceed further it is necessary to ask the question: Is Acromegaly the result of a breakdown of one of the growth mechanisms of the normal body? I think this question must be answered in the affirmative. It is a breakdown of the body's chief adaptational mechanism—the mechanism which "makes the back equal to the burden it has to bear." That a mechanism of this nature must be present in the body, becomes apparent, I think, if one will but mark what happens in the various systems of the body when a man or animal is placed under training. The muscles are the structures chiefly concerned but it would be useless for them to undergo a hypertrophy unless they had some means of calling forth a corresponding growth reaction from heart and vessels so as to be supplied with an increased amount of blood, from the lungs, to meet their increased need of oxygen; from the alimentary system, so as to be supplied with increased energy-material; from the kidneys, so as to rid them of their increased refuse

products, from the skeletal and articular and ligamentous systems, so that they become fit to withstand the increased stresses produced by hypertrophied muscles. These effects cannot be produced in a merely mechanical way; we must postulate an organized system of some kind and the one which answers all the circumstances of the case is that of hormones. In Acromegaly we find a disorderly manifestation of all the results which follow increased use of muscles—bones strengthen, particularly their muscular ridges and impressions; the joint-surfaces enlarge, the ligaments are thickened, the heart, arteries and veins hypertrophy, the lungs and chest undergo a true growth; the apparatus of mastication and the alimentary canal become hypertrophied, renal substance is increased. If we suppose that this adaptational mechanism, which makes hypertrophy answer to increased action, were to be set in motion, not through the normal stimulus of muscular activity but through some condition arising in the pituitary itself, then we can understand why it is that the human body should be subject to such a disorder as Acromegaly.

One other fact supports the theory here put forward regarding the nature of Acromegaly. Why should the hands and feet be the parts of the body which suffer first and most in Acromegaly? If my explanation is the true one, then the feet and hands should be the most susceptible, for nothing is more certain than that hard manual labour will produce marked growth responses in the hands and feet.

THE PHARMACOLOGICAL ACTION OF ADRENALIN ON THE SPHINCTER PYLORI OF THE FOETUS

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Pirie's † recent suggestion that hypertrophic stenosis of the pylorus in newly born infants may be due to fœtal hyperadrenia makes it necessary to view that condition from an entirely new angle.

The development of hypertrophic stenosis according to Pirie comes about as follows:— (1) Reflex stimulation of the adrenal medulla from some internal or external receptor, *e. g.,* the preputial nerve endings, is followed by (2) the discharge of an increased amount of adrenalin into the blood stream.‡ (3) This increased adrenal discharge stimulates the splanchnic and causes, according to whether it is constant or intermittent, either increased tone or spasmodic contraction of the alimentary sphincters (ileocolic and pyloric) because of the so-called reverse innervation of these muscle groups. Hypertrophy of the sphincter pylori follows because of this spasm or increased tone, and persists into post-fœtal life. The cause of stimulation disappears or is persistent only as some minor defect (phimosis) which is not usually recognized as causally associated with the graver pathological result. It may, however, be sufficient to maintain the severity of the morbid condition for which the infant comes under treatment.

Pirie cited cases of babies suffering from hypertrophic stenosis who recovered after circumcision and mentioned the autopsy of a child dead from hypertrophic stenosis at which gross adrenal lesions were found to support this attractive hypothesis. It is obvious that this hypothesis is dependent on the so-called "reversed innervation" of the gastro-intestinal sphincters. In other words, the statement must be accepted that while the augmentor fibres supplying the gastro-intestinal musculature in general reach the digestive tract from the vagus nerve and the inhibitors from the splanchnic, the muscles of the pyloric and ileo-colic sphincters are augmented through the splanchnic and depressed by way of the vagus. Stimulation of the splanchnic nerves (peripheral), according to this theory, results in relaxation of the entire gastro-intestinal musculature except the ileo-colic and pyloric sphincters which are said to be thrown into a state of increased tonus by the impulse following splanchnic excitation.

Almost nothing is known about the behavior of the intestine during intra-uterine life.

It has been generally accepted that peristalsis occurs before birth because lanugo hairs are found in the first meconium. These hairs could only have reached the large intestine by passing through the whole gastro-intestinal tract after having been swallowed with amniotic

† Pirie, G. R., Lancet, September 20, 1919.

‡ In the British Medical Journal of November 26, 1921, (p. 891) Gray and Reynolds have suggested that the hypersecretion of adrenalin may be maternal as well as fœtal.

fluid into which they had been cast off. The general opinion regarding ante-natal intestinal movement has been that peristalsis is feeble and infrequent until just before the birth of the fœtus. Yanese,* indeed, found that in human fœtuses he was able to produce peristalsis and local contractions as early as the ninth week of intra-uterine life. Before this date, when the myenteric plexuses and the longitudinal muscle coat begin to develop, this author claimed to have been able to produce neither peristaltic movements nor local contractions by direct stimulation. He concluded, therefore, that peristaltic movements are of neurogenic rather than myogenic origin.

In the course of a study of the physiology of the gastro-intestinal tract in the fœtus we have observed the action of adrenalin solutions on the musculature of the stomach and intestine, and since the appearance of the hyperadrenia theory of pyloric stenosis, it has seemed worth while to record the reaction of the fœtal sphincter pylori to solutions of adrenalin. Solutions of adrenalin were tested on ring and strip preparations of isolated sphincter pylori and on preparations of the pylorus *in situ*.

Isolated preparations of embryonic pyloric muscle show very characteristic regular spontaneous contractions when kept in oxygenated warm Ringer solution. The graph made by such an isolated ring is shown below (Fig. No. 1). Contractions usually start two or three minutes after the preparation is set up and increase steadily and rapidly in strength until a maximum contraction is reached, which may be maintained for two or three hours. While the contractions are usually singularly rhythmic, the rhythm maintained may be multiple (in groups of two or three beats, Fig. No. 2) and occasionally, in chilled or shocked preparations, the contractions are irregular in rate and force. The character of the curve made by these muscular contractions is strikingly uniform—a rapid contraction indicated by a steep, almost vertical ascending limb, and a rapid initial relaxation slowing to its completion, so that the descending limb of the curve blends gently with the horizontal. Ascent or relaxation may be interrupted by secondary contractions which produce ana- or cata-crotic notches preceding the relaxation of the muscle, or the curve may be topped by a smooth or serrated plateau; double contractions may occur which are followed by a contraction wave of extra height, or by a compensatory pause, a phenomenon which we shall discuss in more detail later.

Now if a solution of adrenalin be instilled into the Ringer bath in which the strip of muscle is working, a pronounced change in the rhythm and force of the contraction takes place which is characteristic and constant for a given dose of the drug. If a small dose of the salt is given (0.0005 mgm. in 50 c.c. of the bath solution), the interval between the contractions is lengthened immediately so that three or four times the usual period may intervene between contractions without any departure of the lever from the base line (Fig. 3b). When the muscle does contract, however, it may do so with somewhat greater force, so that the resulting curve of the contraction wave is very slightly higher than the curve of the contractions immediately preceding the instillation of the adrenalin solution. On the other hand, after a slightly larger dose, the amplitude of the contraction may be diminished, the interval remaining the same, or both amplitude and frequency of contraction may diminish. When the amplitude of the contraction is very much diminished, the return to normal contraction force may be gradual (Fig. 3a). This is maintained until the preparation is washed out or the drug is destroyed by oxidation. This decreased frequency of contraction is more pronounced as larger doses of the drug are given until, following the exhibition of massive amounts (0.3 mgm. in 50 c.c.), the muscle may remain completely paralyzed for half an hour or more, even though the specimen has been twice or three times washed. Return of contractility to such a paralyzed muscle is like the onset of contractions in a fresh, shocked, preparation. The muscle contracts at first to a hardly recordable extent and gradually progressively, minute by minute, the contraction attains its full strength (Fig. 4). The action of adrenalin on pyloric strips is quite the same.

The description of the increase in the size of the contraction following the administration of small doses of adrenalin might make one feel that, besides the depressor action of the drug, some pressor action was present also which increased the contraction of the muscle, but a study of the graphs made from untreated preparations shows that an increase in the strength of contraction is seen following any pause in the course of the spontaneous rhythmic muscular movements or lengthened interval between contractions. It will be seen, therefore, that this apparent increase in contraction is analogous to the heightened response of the cardiac muscle after the removal of the inhibition caused by vagus stimulation.

The above account of the results of splanchnic stimulation holds good not only in the pig fœtus, on which most of our studies have been made, but also in all other mammals which we have studied, including man. Since pyloric stenosis occurs only in man, as far as we at present know, an examination of human fœtal material is necessary in order to exclude the possibility of special peculiarities. This is especially so since human tissue, at least in one

* Yanese, T.: Arch. ges. Physiol., LXVII, 345-383.

instance, is said to vary from that of other mammals in its response to chemical stimulation (see Barbour's description of the response of the human coronary artery to adrenalin). It may be well, therefore, in this connection to give a protocol of one of the experiments on the pylorus of a human fœtus.

The child R. M., female, prematurely born in the sixth month, weight 907 gms., admitted in the eleventh hour of extra-uterine life. The child was said to have grown progressively weaker since birth.

Death at 8.30 A. M., the day following admission.

At 10 P. M. the stomach was opened and found filled with a slightly alkaline fluid consisting of the milk feeding given two hours before death. The milk showed no evidence of coagulation.

The pylorus was normal to inspection. It was cut into strips one of which was immediately suspended in 50 c.c. of oxygenated Tyrode's solution at 39° C. and attached to a light lever.

Strip No. 1. Spontaneous contraction of the muscular strip started immediately and continued with experimentally induced variation until 11.20 P. M., when the preparation was discarded. The instillation into the bath of 0.1 c.c. of a 1 : 100,000 solution of adrenalin chloride (P.D.) (a concentration of 1: 50,000,000) was followed by no other change in the contraction than a slight slowing of the contraction rate. The instillation of double the amount of the drug was equally unproductive of appreciable effect. Following the instillation of 1 c.c. of 1 : 100,000 solution of the drug, however, (1 : 5,000,000) the amplitude of the contraction curve was considerably diminished and the exhibition of 1 c.c. of a 1 : 10,000 solution (1 : 500,000 concentration) caused a relaxation of muscular tone and diminution of contraction amplitude to approximately one-fifth of the normal value, a condition of affairs which persisted until, just before removal of the strip from the bath, stimulation with 0.1 mgm. of histamine restored approximately the pre-experimental muscular vigor (Fig. 5).

Strip No. 2, four hours and 20 minutes after death, 2 hours and 50 minutes after removal from the cadaver, gave an equivalent reaction to that given by preparation I.

Strip No. 2, studied at 3.30 P. M. on the day following death under the same conditions described above, gave only feeble contractions which were marked by inhibited adrenaline chloride 1 : 50,000,000 and which entirely ceased under the influence of the same drug 1 : 5,000,000. This strip was kept at refrigerator temperature in Ringer's solution for 31 hours after death.

It will be seen from the above experiment that the reaction of the human fœtal pylorus differs in no respect from the response to adrenalin given by the pyloric musculature of fœtuses of other mammalian species. This response is in the nature of relaxation and inhibition rather than of stimulation and augmentation.

We have been able to confirm the results given by ring and strip preparations, isolated from the fœtal body and maintained *in vitro,* by experiments in which the pylorus was left *in situ* and the adrenalin was exhibited to the pylorus with the blood and nerve supply uninterrupted.

Increased secretion of adrenalin it would seem, could hardly result in anything else than a decrease in the tone of the pyloric muscle, and intermittent discharges of adrenalin, instead of inducing periodic pyloric spasm, would rather result in periods of relaxation of the gastro-intestinal motor mechanism.

Furthermore, since the action of adrenalin on the muscle of the gastro-intestinal tract depends on stimulation of the endings of the splanchnic, reversal of valvular innervation, if it exists in the adult intestine, must be a phenomenon confined to post-fœtal life.

It is interesting to note that in the pig fœtus, even in those with a C. R. length of only 6 cm., it is possible to see definite peristalsis when the gut is exposed after delivery from the uterus. Fœtal peristaltic movements follow the "law of intestine" as laid down for the adult animal, bear the same relation to the Tonus rings and are affected in the same way by stimulation. Contractions of segmentation occur and are more readily seen than in the adult gastro-intestinal tract and, in brief, intestinal motor mechanism has the potentiality to carry out the movements described as characteristic for the gastro-intestinal tube in post-embryonic life.

FIG. 1.—Spontaneous contractions of an isolated ring of embryonic pyloric muscle (pig).

FIG. 2.—Spontaneous contractions of a ring of pyloric muscle from a pig embryo, to show compound contractions and multiple rhythm in a cooled preparation. Temperature of the bath 36° C.

FIG. 3.—*a* and *b* show the effects of small doses of adrenalin chloride on the contractions of rings of embryonic pyloric muscle (pig) suspended in 50 c.c. of Ringer-Locke solution. While the contraction rate is much slower after very many doses of adrenalin, the tone of the muscle remains unchanged. Recovery may be sudden or gradual. During the period of recovery the amplitude of the contractions may return to normal or slightly above, while the frequency is still diminished. The immediate effect of the drug is the diminution of both amplitude and frequency of contraction.

FIG. 4.—Shows complete cessation of contractions of a ring of pyloric muscle of a pig embryo after 0.3 mgm. of adrenalin chloride was added to a bath of 50 c.c. Ringer-Locke solution.

FIG. 5.—Curve showing the effect of adrenalin chloride on a preparation of the pyloric muscle of a human fœtus which contracted spontaneously in 50 c.c. of Ringer-Locke solution. This curve shows that the tone of the muscle was impaired by the drug as well as the frequency and amplitude of the contractions.

FIG. 1.

Spontaneous contraction of a ring of pyloric muscle. Pig fœtus C. R. 115 mm. Ringer-Locke solution.

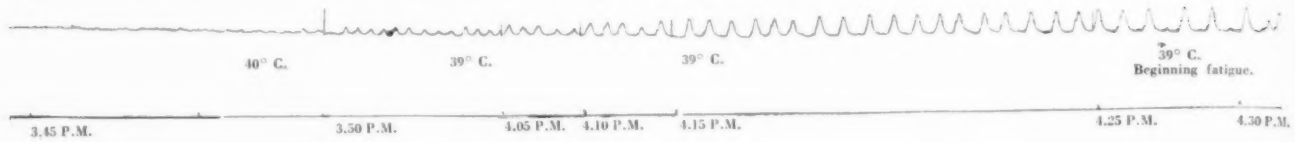


FIG. 2.

Compound contractions and multiple rythm of a ring of pyloric muscle induced by cooling. Pig fœtus C. R. 146.1 mm.

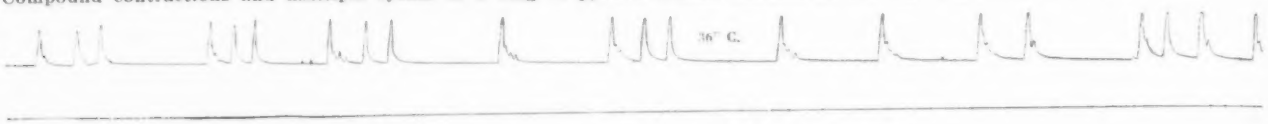


FIG. 3a.

Pylorus of a pig fœtus. C. R. 157 mm. in 20 c.cm. of Ringer-Locke solution.

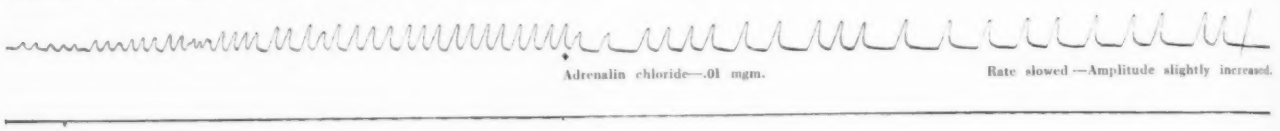


FIG. 3b.

Effect of Adrenaline Chloride on pylorus of a pig embryo. C. R. 171 mm. Ringer-Locke. 38° C.

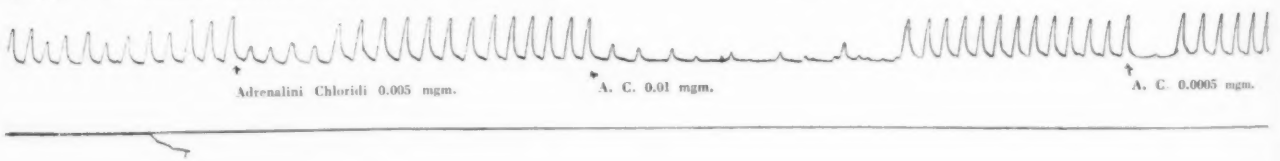


FIG. 4.

Effect of adrenaline chloride on the spontaneous contractions of the pylorus. Pig embryo C. R. 155 mm. Ringer-Locke solution. 20 c.cm.

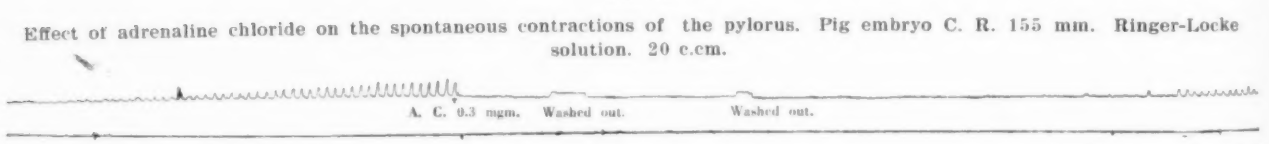
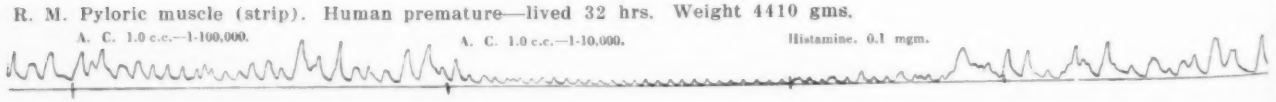


FIG. 5

R. M. Pyloric muscle (strip). Human premature—lived 32 hrs. Weight 4410 gms.



OCCURRENCE OF ANAEMIA IN RATS ON DEFICIENT DIETS

By William M. Happ

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Introduction. Numerous attempts have been made to produce anæmia in animals by means of diets poor in iron. In these experiments attempts have often been made to ascertain whether inorganic iron given by mouth causes the regeneration of the blood in anæmia. Meyer [1] summarized the literature on this subject in 1906.

Hall [2] fed white mice on the following diets:

1.	Casein	37.8
	Starch	28.3
	Fat	30.1
	Salts*	3.7
2.	Casein	20.0
	Butter	3.34
	Fat	11.66
	Cellulose	0.7
	Starch	60.5
	Salts	3.7

He found that the total iron content of the ash of the mice kept for three weeks on these diets decreased 40 per cent. The feeding of iron by mouth increased the number of red blood cells and hæmoglobin. The ingredients of these diets were purified foodstuffs and relatively free from iron. The nature of the fat used was not stated. The animals lost rapidly in weight and lived only four weeks on diet No. 1 and six weeks on diet No. 2. No water soluble *B* was supplied in these diets.

Schmidt [3] was not able to produce anæmia in the first generation of white mice fed on a diet poor in iron. The blood of their young, however, showed a reduction of hæmoglobin and red blood cells, with poikilocytosis, anisocytosis and polychromasia. When iron was added to the diet, the blood became normal. Enlargement of the spleen was noted in these anæmic mice.

Other authors, Kunkel,[4] Cloetta,[5] Abderhalden,[6] Müller,[7] Tartakowsky,[8] and others, fed animals on diets of milk, milk and bread, and milk and rice, to study the regeneration of the blood following bleeding and the effects of the administration of inorganic iron. These experiments showed that a reduction in the amount of iron bound in the tissues (reserve iron) and in the circulating blood (circulating iron) resulted in animals fed on such diets. The depletion could be made up by feeding iron by mouth either in inorganic or organic combination. They showed further that the reserve iron was depleted before the amount of iron circulating in the blood as hæmoglobin was diminished. There have been few studies other than those regarding the hæmoglobin content of the blood of animals on deficient diets.

It is a fact that the prolonged feeding of milk alone to an infant may be followed by the appearance of a secondary anæmia. Because of the work of Bunge [9] and Abderhalden [6] this anæmia has been attributed to a depletion of the reserve store of iron with which these infants are born, and a consequent reduction in the circulating iron (hæmoglobin). Again, there are anæmias associated with rickets in infancy in which a peculiar symptom complex is present, associated with various qualitative and quantitative changes in the blood picture. Children with these anæmias usually have an enlargement of the spleen, which is occasionally extreme, and a slight enlargement of the liver and lymph nodes, and a secondary anæmia with or without leucocytosis. There is often a lymphocytosis, and immature cells of both red and white blood cell series are found in the circulating blood (normoblasts, megaloblasts, myelocytes, pathological lymphocytes, etc.). This clinical picture has received various titles, such as "von Jaksch's anæmia," "anæmia pseudoleukæmica infantum" and "splenic anæmia of infants." It is in all essential respects a secondary anæmia. The frequency of its association with rickets has been emphasized by practically every author on the subject (see review by Evans and Happ).[10] The cause of this anæmia has not been established. Anæmia of this type appears to depend upon more factors than does the simple secondary anæmia which results from a diet low in iron, such as a prolonged milk diet.

On account of the frequency of anæmia in infants with rickets the question arose as to the existence of anæmia in rats made rachitic by faulty diets. Through the kindness and co-operation of Dr. McCollum, Miss Simmonds and Doctors Shipley and Park, I was able to study the blood of rats on various faulty diets. These authors have found that if growing rats are placed on diets in which certain fats, butter fat, cod-liver oil, etc., are absent, or present only in small quantity, and in which there is a certain disproportion between the amounts of calcium and phosphorus, lesions of the bones result which are analogous to the lesions of the bones of young children with rickets.[11]

The result of the examination of the blood of rats on various faulty diets is given, together with the diet employed in each instance.

Technic. Klineberger and Carl,[12] and later Powdermaker,[13] found that the number of cells in blood obtained from the tails of rats was subject to great variation. For this reason the following procedure was employed in obtaining blood for

* Composition of salt mixture.

K_2CO_3	0.97
NaCl	0.7
$CaHPO_4$	1.8
$MgCl_2$	0.18

counting. The rat was lightly anesthetized and laid on a board, ventral side up. The hair over the thigh was cut close. The femoral (superficial) vein, which lies just beneath the skin, was exposed. This vein is quite prominent, especially in albino rats. It was cut with sharp scissors. In this way sufficient blood could be obtained with insignificant trauma for a red and white blood cell count, hæmoglobin determination, a cresyl blue preparation for reticulated red blood cell count, and smears for a differential blood count. As a rule, the flow of blood stopped promptly, and if the animal was to be kept, a drop of collodion was placed on the wound. The counts given in this paper, however, are the results of the first examination, unless otherwise stated. No animal was bled previous to the making of the blood count, the results of which are given. The red and white blood cell counts were done in the ordinary manner, Türck's and Hayem's solutions being used for diluent, and the blood was counted in a Karl Zeiss hæmocytometer with Neubauer ruling. The hæmoglobin determinations were made with the Sahli apparatus. The hæmoglobinometer standard was so prepared that it gave a reading of 95 per cent for blood from the normal human adult. A reading of 95 per cent indicated approximately 14.25 grams of hæmoglobin per 100 c.c. of blood. The blood films for differential counts and for the counting of the reticulated red blood cells were made by the following technique: A clean glass pipette of small bore or a white blood cell pipette was rinsed with a saturated solution of sodium oxalate, the fluid withdrawn and the inside of the pipette thoroughly dried. Several drops of blood from the incised thigh vein were drawn up into the pipette and blown in and out several times on a watch-glass. In this manner the blood was kept from clotting. A film was made from this oxalated blood in the usual manner. These films were stained, when dry, with Wilson's modification of Romanowsky's stain, mounted on a slide, a differential count of the white blood cells was made and the nature of the red blood cells noted. Then a drop of the oxalated blood was drawn into the pipette together with a drop of 1 per cent cresyl blue in normal salt solution. The blood and the stain were well mixed by blowing the contents on a watch-glass. A drop of this mixture was then blown on a cover-slip, films were made and stained with Wilson's modification of Romanowsky's stain, dried and mounted. By this method a preparation was obtained which showed beautifully the reticulum in the red blood cells. These preparations were permanent. Films kept for one year in this laboratory still show the reticular stain of the red blood cells. The percentage of reticulated cells was determined by counting a certain number of red blood cells, 500 or 1000, and recording the number of cells showing reticulum.

Cages. In these experiments three kinds of cages were employed. They are designated as

1. Galvanized iron cages. The iron wire was well insulated by galvanized metal.

2. Wood and iron cages. These are standard cages used in Dr. McCollum's laboratory. They consist of a wooden frame with galvanized iron-wire sides.

3. Wooden cages, made entirely of wood and glass.

Food Cups. Zinc cups were employed in the first two types of cages and glass cups in the third.

Drinking Water. The stock rats were given tap-water, the remaining rats were given doubly distilled water. This was first distilled through a copper still and then redistilled through glass. Animals receiving such water were given one drop per week per animal of the following solution:

Iodine	2 grams
KI	5 grams
H_2O	500 c.c.

The normal blood of the rat. Klineberger and Carl[12] give the following average counts for the blood of the rat (blood from thigh vein):

R. B. C. ...9,300,000	P. M. N. .....16.
Hb. ....105% (Sahli)	P. M. E. 3.5
W. B. C. 15,200	L. lymph 24.5
	S. lymph. 53.5
	L. M. 0.5
	T. ..... 2.
	100.0

These authors found no difference in the blood of the two sexes. The red blood cells of normal animals showed some anisocytosis and polychromasia. The leucocytes were predominantly small lymphocytes. The platelets tended to group themselves in masses.

Donaldson[14] gives the following average counts for albino rats on a "scrap" diet.

R. B. C....7,600,000—9,200,000	P. M. N...44 to 71%
Hb. .....85% to 100%	P. M. E.... 0 to 3%
W. B. C. .....7,200 to 16,000	P. M. B... 0
	Lymph. ...30 to 55

Neither the source of the blood nor the type of the hæmoglobinometer used is stated.

Geiling and Green,[15] in a preliminary report of some studies on blood regeneration in the rat on various diets, give the normal red blood count as between 7.5 and 10.5 millions and the hæmoglobin percentage as 110 to 140 (Smith-Cohen). They state that the number of blood cells per c.mm. in rats of different ages and sexes falls within these limits.

In discussing the blood of normal rats several factors should be taken into consideration: first, the species of rat used; second, the diet given as the stock diet; third, the presence of respiratory infections which are common in laboratory rats and which may modify the total leucocyte count and differential formula; and fourth, the source of the blood. White blood cell counts made on blood from the end of the tail are higher than counts made from venous blood and show a higher polymorphonuclear percentage. I also found that blood from the heart contained a uniformly lower white blood cell count than blood from the veins. The number of red blood cells and the percentage of hæmoglobin, however, were the same in blood from both these sources. It was necessary in some of the small rats to draw the blood from the heart, as a free flow could not be obtained from the cut vein. It should be remembered that the white blood cell counts are lower in these cases than if they had been made on blood from the thigh vein.

In our series of experiments the rats used were hybrids of the albino and the black Norway rat.

The diet employed as a stock diet for our animals was as follows:

Whole ground wheat	
Cracked corn	
Ground rolled oats	āā 3 parts
Flaxseed meal	1 part

This was given *ad libitum* and in addition the rats received a small quantity of pasteurized milk and tap-water daily.

CHART I.

BLOOD COUNTS OF NORMAL RATS AT DIFFERENT AGES.

AGE	Blood from	Hb. %	R. B. C.	W. B. C.	Reticulated R. B. C. %	DIFFERENTIAL P. M. N.	P. M. E.	Lymph.	LM. T.	U.	REMARKS
Newlyborn*	Heart	75	3,200,000	4,000	93	24	2	30	8	8	Myelocytes 4. Nucl. R.B.C. 250 in counting 100 W.B.C. Anisocytosis. Polychromasia corresponding to percentage of Retic. R.B.C. See photograph.
Newlyborn				4,000		43	0	17	5	32	Myelocytes 2. Nucl. R.B.C. 280 in counting 100 W.B.C.
15 Days	Heart	—	—	1,800	19	5	0	95	0	0	No Nucl. R. B. C. Marked leucopenia.
20 Days	Heart	—	—	3,600	20	2	0	90	8	0	No Nucl. R. B. C.
	Thigh vein	80	4,800,000	4,800	—	22	0	76	2	0	Rarity of P. M. N. forms.
26 Days	Thigh vein	85	5,120,000	3,600	18	12	0	76	4	8	
1 Mo.	Thigh vein	80	7,000,000	4,200	—	16	0	76	8	0	
	Thigh vein	85	6,592,000	9,000	6	24	0	74	2		
2 Mos.	Thigh vein	90	7,680,000	8,000	9	16	0	84	0	0	
4 Mos.	Thigh vein	110	11,200,000	9,600	3	24	6	68	2	0	
9 Mos.	Thigh vein	115	10,800,000	8,000	1	26	4	68	2	0	
10 Mos.	Thigh vein	110	9,856,000	12,000	—1	14	0	85	1	0	

* Under newly born are included rats under 24 hours old.

Chart I shows the results obtained in rats of different ages. The blood of a young rat differs from that of the adult, in that in the former the hæmoglobin content and the red blood cell counts are relatively lower, the white blood cell count is lower (a relative leucopenia) and the percentage of lymphocytes is relatively higher. Therefore, it is important in studying the blood of a rat of known age to compare it with a normal for its age. The normal count for a young rat would indicate a slight anæmia for an adult rat. We may, therefore, speak of a physiological anæmia in the nursing rat.

The diets which were used in these experiments may be roughly divided into two groups. The first of these included simple diets which had milk in one form or another as a basis. The second group includes the more complex mixtures of various purified foodstuffs. Rats were fed on

1. Pasteurized milk.
2. Pasteurized milk and bread.
3. Evaporated milk (Pet Brand).
4. Condensed milk (Eagle Brand).
5. Dried skimmed milk (Krystallak).
6. Dried skimmed milk with various additions.

A diet of pasteurized milk alone will not cause pathological changes in the blood of rats, and rats may be reared to the third generation on diets of milk and bread without developing anæmia.

Neither evaporated milk (Pet Brand) nor condensed milk (Eagle Brand) induced anæmia in rats even when the intake was limited to 8 c.c. and 4 gm., respectively, per diem in order to restrict the growth of the animals.

The attempt to cause anæmia by diets of dried skimmed milk (Krystallak) was a failure. Nor was anæmia produced by diets which contained Krystallak with yeast, butter fat or bread, or any combination of these additions to the skimmed milk diet. When, however, casein was added to a ration of Krystallak and butter fat, an anæmia was produced in two animals.

A. A female rat was started on a diet of

Krystallak	95
Butter fat	5
	100

at the age of 25 days, when she weighed 30 grams. She was on the diet five months, during which time she gained in weight to 130 grams. At this time casein was substituted for 15 per cent of Krystallak:

Krystallak	80
Casein	15
Butter fat	5
	100

The animal was kept in a galvanized iron cage on this diet for four weeks and lost 30 grams in weight. The rat appeared pale. Blood was taken from a thigh vein:

R. B. C. 1,920,000
Hb. .....20% Leucopenia too marked
W. B. C. .....1,600 for accurate differential.

Red cells showed slight anisocytosis and poikilocytosis and there were many nucleated red cells. Polychromasia was fairly well marked. Approximately 70 per cent of the white cells were lymphocytes.

Autopsy. The tissues were very pale. The spleen was normal in size. The bones appeared normal and cut with resistance. Lungs normal.

B. A male rat, which was in cage with the above rat, was put on the same diet at the age of 25 days, when weighing 30 grams. He was on the diet five months, during which time he gained in weight to 150 grams. Fifteen per cent of casein was added, and the rat was kept on this diet for 6

weeks, on which he lost 20 grams in weight and developed pallor. Blood from thigh vein:

R. B. C. ...1,472,000	P. M. N. 51
Hb .....15—20%	Metamyelocytes 5
W. B. C. 5,600	P. M. E. 5
	Eosinophilemyelocytes. 1
	Lymph. 27
	T. 1
	Unclassified 10
	100*

67 nucleated red cells were seen in counting 100 W. B. C. There was slight anisocytosis and poikilocytosis. Polychromasia was well marked.

Autopsy. As for above rat.

Microscopic sections: Bone. The cartilage was not calcified. There was a narrow subchondral zone of irregular trabeculæ with a great deal of osteoid tissue. The cortex of the shaft was thickened and spongy. The marrow was congested.

The following types of complex diets were also studied to determine if possible the relations of certain deficiencies and excesses to the production of anæmia.

7. Purified diets (*a*) without iron, (*b*) with iron.
8. Diets low in fat-soluble *A*.
9. Diets low in calcium.
10. Diets low in calcium but with an excessive amount of fat-soluble *A*.
11. Diets low in water-soluble *B*.
12. Diets low in phosphorus and fat-soluble *A*.
13. Diets low in calcium and in fat-soluble *A*.
14. Other diets which produced changes in the bones.

7. *No anæmia resulted in the first generation and only slight anæmia in the second generation from a purified complete ration without added iron.*

For this series of animals a purified diet was used (indicated below as purified diet without iron).

	Salt mixture.	
Washed casein ..25**	NaCl	0.173
Butter fat 10	$MgSO_4$	0.266
Agar 2	K_2HPO_4	0.954
Wheat germ.....10	$CaH_4(PO_4)2$..	0.540
Salt mixture..... 3.58	Ca Lactate...	1.300
Washed dextrin..qs. ad 100	NaH_2PO_4	0.347
		3.580

* In the formulas of the differential counts.
P. M. N. = polymorphonuclear neutrophile leucocytes.
P. M. E. = Eosinophile.
Lymph. = Lymphocytes (large and small).
T. = Transitional monocytes.

** I take pleasure here in thanking Dr. McCollum for outlining and analyzing for me this and the other complex diets which were used.

† The diets of the mother rats were never changed during lactation, that is, if the mother of a litter was on a deficient diet, she continued to receive it while the young were being nursed. The second generation animals received the same food as their parents, unless otherwise stated. Animals of the second generation on a faulty diet may be said, therefore, to have received the deficient food since birth, although it has been shown that the breast milk of a mother on a deficient diet is a better food for her young than the diet she receives is for her.

For the control series the identical diet (indicated below as purified diet with iron) was used except that ferrous lactate 0.118 grams was added to the salt mixture, bringing the salt mixture to 3.7 instead of 3.58%.

Both of these groups of rats were kept in wooden cages with paper for bedding and with glass doors and glass food and drinking cups. They did not come in contact with iron.

7a. Purified diet without iron.

A. A mother rat was put on the purified diet without iron at the birth of her young and remained on it while she nursed them.† Blood was examined from thigh vein of a male rat of this litter which had been on the same diet for 2½ months. His weight at this time was 250 grams.

R. B. C. 12,240,000	P. M. N. 18
Hb. 115%	Lymph. 70
W. B. C. 12,000	T. 2
Reticulated R. B. C..1%	Unclass. ..... 10 (smudge cells)
	100

At the age of 2½ months a splenectomy was performed under ether anesthesia. The spleen was larger than normal and weighed 0.91 grams. The animal recovered promptly and was kept 2½ months longer on this diet; weight 320 grams (total of 5 months on a diet); blood from thigh vein:

R. B. C. 9,920,000	P. M. N. 7
Hb. 110%	P. M. E. 6
W. B. C. 40,000	Lymph. 84
Reticulated R. B. C...3%	To 3
	100

This animal had a severe respiratory infection.

Microscopic examination of the spleen showed a simple hypertrophy.

Autopsy. Subcutaneous fat abundant. The tissues were of good color. There was no infection about the wound and no hypertrophy of the lymphatic tissue. The bones were hard and normal in appearance.

B. A male rat of the same litter as the preceding rat on the same diet five months, but no splenectomy performed; weight 280 grams, blood from thigh vein:

R. B. C. 12,300,000	P. M. N. 11
Hb. 1.15%	P. M. E. 6
W. B. C. 12,800	Lymph. 78
Reticulated R. B. C. 3%	T. 3
	Unclassified 2
	100

Autopsy. The spleen was larger than normal, weighing 0.85 grams. Autopsy findings as in preceding rat.

7b. Purified diet with iron.

A. Control rat for the above. The mother of this rat was put on a purified diet with iron at birth of the young. A male rat of her litter was on this diet for 2½ months. Weight 250 grams. Blood counts at this time from thigh vein gave the following result:

R. B. C. 11,872,000	P. M. N. 14
Hb. 115%	P. M. E. 2
W. B. C. 15,000	Lymph. 76
Reticulated R. B. C. 1-2%	T. 4
	Unclassified. 4
	100

A splenectomy was performed under anesthesia; the weight of the spleen was 0.52 grams. The animal made a good recovery and was kept on this diet for 2½ months longer, making a total of 5 months on a diet. Weight 310 grams. Blood from thigh vein:

R. B. C.	9,920,000	P. M. N.	29
Hb.	115%	P. M. E.	9
W. B. C.	12,400	Lymph.	60
		T.	2
			100

Microscopic examination of the spleen showed it to be normal.

Autopsy. Entirely negative, wound perfectly healed.

B. A male rat of the same litter as *A*, on the same diet for five months. No splenectomy performed; weight 290 grams; blood from thigh vein was counted as follows:

R. B. C.	12,640,00	P. M. N.	10
Hb.	115%	P. M. E.	1
W. B. C.	14,400	Lymph.	84
		T.	5
			100

Autopsy. The spleen weighed 0.62 grams. Examination entirely negative.

The blood of two other rats of this series was examined with practically the same results. The blood of rats of the first generation on a purified diet without iron was found to be practically identical with that of rats on the same diet with iron added. The spleen of rats on the former diet, however, was uniformly larger than the spleen of those on the latter diet. Splenectomy had no effect on the blood picture.

Slight anæmia resulted in some of the animals in the second generation receiving the purified diet without iron.

A. A female rat, whose mother had been on the purified diet without iron since her birth, at the age of 19 days weighed 21 grams. The blood of the mother was normal after the weaning of the litter. She had been on the deficient diet during lactation. Blood from thigh vein:

R. B. C.	4,112,000	P. M. N.	32
Hb.	45%	Lymph.	64
W. B. C.	3,400	T.	4
Reticulated R. B. C.	15-20%		100

No nucleated red cells were seen. There was slight anisocytosis, no poikilocytosis, but fairly marked polychromasia.

Autopsy. Spleen normal in size, weight 0.77 grams, tissues somewhat pale, bones cut with resistance.

B. A young rat, whose mother had been on a purified diet without iron since her birth, at the age of 42 days weighed 29 grams. Blood from thigh vein:

R. B. C.	6,400,000	P. M. N.	40
Hb.	60%	Lymph.	60
W. B. C.	4,600	T.	0
Retic. R. B. C.	15%		100

The red cells showed moderate polychromasia, otherwise they were normal.

Autopsy. The spleen was small, weighing 0.1 gram. Liver and tissues fairly good color. Lungs normal. The bones cut with resistance.

C. One of the young of a mother rat which had been on a purified diet without iron since birth, at the age of 24 days, weighed 25 grams. Blood from thigh vein:

R. B. C.	7,360,000	P. M. N.	28
Hb.	67%	Lymph.	72
W. B. C.	7,600	T.	0
Retic. R. B. C.	10%		100

Slight aniso- and poikilocytosis and polychromasia. No nucleated R. B. C. seen. No autopsy.

D. A young rat of the same litter as the preceding but aged 2 months. Weight 110 grams. Blood from thigh vein:

R. B. C.	not done	P. M. N.	14
Hb.	100%	Lymph.	86
W. B. C.	5,600	T.	0
Retic. R. B. C.	8%		100

Slight polychromasia, red cells otherwise normal in appearance.

Autopsy. Tissues and viscera of good color. The spleen was slightly enlarged, the thymus persistent and bones cut with resistance.

E. Mother rat was put on purified diet with iron at birth. Blood of mother normal after weaning litter. One female of this litter at the age of 19 days weighed 32 grams. Blood from thigh vein:

R. B. C.	6,144,000	P. M. N.	10
Hb.	80%	Lymph.	88
W. B. C.	3,400	T.	2
Retic. R. B. C.	10%		100

There was a slight anisocytosis and polychromasia. No nucleated red cells were seen.

Autopsy. The spleen was normal in size, weighing 0.1 gram; the thymus somewhat enlarged. The bones were hard and cut with resistance.

F. Rat, aged 1 month, weight 40 grams. Third generation on purified diet with iron. Blood from thigh vein:

R. B. C.	10,240,000
Hb.	110%
W. B. C.	2,600

The red cells appeared normal and the differential blood picture was essentially normal.

No autopsy.

7a. *Diet 2.*

Casein	12.5
Butterfat	10.0
Wheat germ	5.0
Agar	2.0
Salt mixture without iron	3.7
Dextrin	67.0
	100.0

The diet was made of purified food stuffs. It was iron free and had about half the protein content of the last mentioned diet. Two generations of rats were reared on this diet. Four rats of the second generation, weighing 33 to 40 grams, were kept on this diet from the age of 30 days for three months. At the end of this period they weighed 130 to 150 grams. on purified diet with iron. Blood from thigh vein:

R. B. C.	10,880,000
Hb.	90%
W. B. C.	7,000

The smears showed red blood cells which were apparently normal. The differential count was normal.

Autopsy. Tissues and viscera of good color. The spleen was enlarged; the bones appeared to be normal.

Microscopic sections of the bones of rats raised on the above purified diets showed practically normal bones. The cartilage was well calcified but the trabeculæ were somewhat fewer in number than normal, *i. e.*, the bones were slightly osteoporotic.

8. *The blood of rats on a diet so deficient in fat soluble A as to produce xerophthalmia showed no anæmia.*

The diet which was used had the following composition.

Rolled oats	40.0
Flaxseed oil meal	8.3
NaCl	1.0
$CaCO_3$	1.5
Dextrin	49.2
	100.0

9. *The following diets were adequate in all respects except for a deficiency in calcium.*

Wheat	20.0
Maize	15.0
Rice	9.5
Rolled oats	9.5
Peas	10.0
Navy beans	10.0
Casein	10.0
While milk powder	5.0
NaCl	1.0
Butter fat	10.0
	100.0

Wheat	20.0
Maize	19.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
$NaHCO_3$	1.5
Butter fat	10.0
	100.0

The blood of rats fed on these diets was quite normal.

10. *This was also true of the blood of rats which were kept on the following diet which contained an excessive amount of calcium and fat.*

Wheat	20
Maize	10
Rice	9
Rolled oats	9
Peas	9
Navy beans	9
Casein	10
NaCl	1
$CaCO_3$	3
Butter fat	20
	100

11. *Rats on a diet so deficient in water soluble B as to produce polyneuritis were not anemic but showed a leucopenia with evidences of diminished leucopoietic activity.*

The diet had the following composition:

Casein	18
*Salt mixture No. 185	3.7
Agar	2.0
Butter fat	5.0
Dextrin	71.3
	100.0

A. A male rat was put on this diet, at the age of 55 days, when weighing 70 grams. At the end of 137 days he weighed 65 grams. In a state of polyneuritis. Blood from thigh vein:

R. B. C.	10,320,000	P. M. N.	19
Hb.	100%	Lymph.	35
W. B. C.	2,600	T.	1
			55

The red cells appeared normal, the polymorphonuclear cells were chiefly cells with 5 or 6 lobed nuclei. The nuclei often filled the entire cells.

B. A male rat, weighing 56 grams, was put on diet at the age of 50 days. At end of 114 days weighed 50 grams. Animal in a state of polyneuritis. Blood from thigh vein:

R. B. C.	10,000,000
Hb.	110%
W. B. C.	1,800

The smears showed very marked leucopenia; the red cells appeared normal. The white cells were about equally divided between polymorphonuclears and lymphocytes. The polymorphonuclear cells had multilobulated nuclei.

Autopsies. These rats with polyneuritis were poorly nourished rats. The spleens were of normal size. Thymus glands atrophic. Tissues of good color and the bones hard on section.

Microscopic section of the bones of these animals showed that the cartilage was thin and well calcified. Few trabeculæ were visible. The marrow was hæmorrhagic.**

Findlay[16] described the same changes in the blood in beriberi in man as we have found in rats, namely, leucopenia and a shift to the right in the Arneth formula without important changes in the red cells and hæmoglobin.

12. *Diets which are relatively high in calcium but which are low in phosphorus and in an uncharacterized substance which is present in certain fats do not produce anæmia in rats.*

McCollum, Simmonds, Shipley and Park have described a very severe form of rickets which is produced by diets which have the above described characteristics. The animals whose blood was studied were fed on their diet No. 3143 which was made up as follows:†

Wheat	33.0
Maize	33.0

* Salt mixture No. 185 is the same as the salt mixture above in the purified diet without iron.

** The pathological condition of the bone and bone marrow of these rats with polyneuritis is described by Shipley, McCollum and Simmonds, Jour. Biol. Chem., Dec., 1921.

† The blood counts on these animals were made by Dr. A. A. Weech.

Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

The blood of four animals on this diet was studied. The diet did not produce anæmia in any case and the leucocytes were quite normal.

13. *Diets low in calcium, normal or relatively high in phosphorus and low in a substance contained in certain fats caused bone changes in rats which were closely related to rickets. These diets also produced anæmia in both first and second generations.*

A brief review of the work of Shipley, Park, McCollum and Simmonds may serve to make the following discussion clearer. These authors have found that on diets in which an organic substance, present in cod-liver oil and to a less extent in butter fat, which may or may not be identical with fat soluble *A*, is small in amount and in which there is a disproportion between the calcium and phosphorus, a condition of the bones results which is practically identical with human rickets. Park and Shipley have frequently noted in these animals varying degrees of enlargement of the spleen and marked pallor of the viscera particularly of the liver. The rats whose blood counts are recorded below were from their series and had been in cages made of wood and galvanized iron. The blood of rats on six diets differing in composition but with the same faults were examined.

Six rats, aged 45 days, whose average weight was 45 grams, were put on the following diet which contains a poor fat and an insufficient quantity of calcium but an abundance of phosphorus:

Wheat	30.0
Maize	19.5
Peas	8.5
Rolled oats	8.5
Rice	9.5
Navy beans	8.5
Casein	10.0
NaCl	1.0
$NaHCO_3$	1.5
Cottonseed oil	3.0
	100.0

A. One female was examined at the beginning of the experiment. Blood from her thigh vein was examined with the following result:

R. B. C.	7,928,000	P. M. N.	9
Hb.	92%	Lymph.	82
W. B. C.	4,700	T.	9
			100

Slight polychromasia, no anisocytosis or poikilocytosis.

B. A male was examined after receiving the diet for 3 months. Blood from his thigh vein showed:

R. B. C.	10,208,000	P. M. N.	22
Hb.	100%	P. M. E.	2
W. B. C.	14,400	Lymph.	74
Retic. R. B. C.	8%	T.	2
			100

Slight polychromasia and anisocytosis were present.

Autopsy. The spleen was of normal size. The tissues were of good color. The thorax was much deformed.

Microscopic sections. There was a great overproduction of osteoid tissue in the epiphysis and the shaft was irregularly calcified and was invaded by blood vessels. There were large numbers of trabeculæ in the shaft each surrounded by a broad zone of osteoid. The cortex of the shaft was thickened. The spaces between the trabeculæ near the marrow cavity contained large numbers of basophilic cells. The spleen showed marked congestion. The Malpighian bodies were larger than normal.

C. A female was examined after being on the diet for 4½ months. She weighed 84 grams. Blood from thigh vein showed

R. B. C.	9,600,000	P. M. N.	10
Hb.	110%	Lymph.	88
W. B. C.	9,000	T.	2
Reticulated R. B. C. less than 10%.			100

The red cells showed slight polychromasia, otherwise they were normal.

Autopsy. The spleen was slightly enlarged. The thorax was deformed and the costo-chondral junctions were enlarged. The bones cut easily and showed broad metaphyses.

Microscopic section. The changes in the bones were similar to those just described. The Malpighian bodies of the spleen were enlarged and contained nests of erythropoietic cells.

D. A female was examined after being on the diet for 6 months. The animal was pale and weighed 80 grams. Blood from thigh vein:

R. B. C.	2,608,000	P. M. N.	37
Hb.	25%	Lymph.	61
W. B. C.	12,000	T.	2
Retic. R. B. C.	50%		100

Eighteen nucleated red cells were seen in counting 100 white cells. There was a marked polychromasia. The polychromatic red cells corresponded with the number of reticulated red cells (see Figs. II, III, IV). Anisocytosis and poikilocytosis were fairly well marked.

Autopsy. The spleen was large, weighing 0.8 grams. The liver and other tissues were very pale. There was marked rickets. The bones cut easily and showed broad metaphyses.

Microscopic section. The bone showed more extensive rickets than in the preceding animal. There was no calcification of cartilage, which was very irregular (see Figs. V and VI).

The Malpighian bodies of the spleen were fewer in number than in the spleen of the preceding rat. There was infiltration with round cells and large mononuclear cells. There was myeloid metaplasia of the spleen with evidences of the assumption of hematopoietic function.

Summary. A rat fed upon a defective diet for 6 months had severe rickets, splenomegaly, and actively regenerating secondary anemia.

E. A rat of another litter fed upon the above defective diet from birth at age of 2 months weighed 30 grams. Blood from the thigh vein:

R. B. C.	7,360,000
Hb.	110%
W. B. C.	2,200
Reticulated R. B. C.	10%

The red cells showed slight polychromasia. There was leucopenia with a normal differential formula.

Autopsy. The spleen was normal. There were signs of early rickets.

F. A female rat, at the age of 40 days, when weighing 55 grams, was put on the following diet which is deficient in calcium and fat:

Wheat	25.0
Maize	19.5
Rice	9.5
Rolled oats	9.5
Peas	9.5
Navy beans	9.5
Casein	10.0
NaCl	1.0
Dextrin	1.5
Whole milk powder	5.0
	100.0

At the end of 7½ months her weight was 83 grams. Blood from the thigh vein contained:

R. B. C.	5,232,000
Hb.	60%
W. B. C.	10,000
Reticulated R. B. C.	24%

The red cells showed moderate polychromasia, marked anisocytosis and some poikilocytosis. A few nucleated red cells were seen. About 75 per cent of the white cells were lymphocytes. There were no myelocytes in the blood film.

Autopsy. The thorax was much deformed. The spleen was slightly enlarged, weighing 0.55 gram.

Microscopic section. The bones showed rickets in process of healing. The spleen showed evidences of hematopoiesis.

Summary. A moderate anæmia for a rat of this age with active regeneration of the red cells. Slight enlargement of the spleen. Rickets.

A. A female rat, aged 45 days and weighing 65 grams, was fed upon the following diet, which is also deficient in calcium and fat:

Soy bean	30
Wheat gluten	5
Casein	10
Wheat germ	5
NaCl	1
Maize	20
Dextrin	29
	100

Her weight was 75 grams at the end of 78 days. Blood from thigh vein:

R. B. C.	3,120,000	P. M. N.	22 (incl. 2 metamyelocytes)
Hb.	35%		
W. B. C.	13,000	P. M. E.	0
Retic. R. B. C.	75-80%	Lymph.	78
			100

Seventy-five nucleated red cells were seen in counting 100 W. B. C. The red cells showed anisocytosis and poikilocytosis and marked polychromasia (see Figs. VII and VIII).

Autopsy. The spleen was very much enlarged. There were marked changes in the skeleton. The cartilage was redundant. Calcification was very defective and irregular. The metaphysis consisted of osteoid tissue in the form of trabeculæ, somewhat irregularly arranged. At the periphery of the bone, processes of unchanged cartilage ran down from the cartilage into the metaphysis. There were small islets of cartilage cells in the metaphysis. The cortex of the shaft was spongy. There was a great deal of osteoid tissue around all of the trabeculæ in the spongiosa. There were no signs of abnormal resorption in the metaphysis but resorption was going on actively in the cortex and in the spongiosa near the marrow cavity (see Figs. IX and X).

Summary. Rachitic rat; enlarged spleen; severe secondary anæmia; evidences of active regeneration of red cells.

13. *Anæmia was caused in the second generation of rats fed upon the following diets that produced changes in the bones.*

A. A male rat, aged 41 days, weighed 22 grams. The mother was on the following diet for 217 days before birth of her litter:

Wheat	19.3	
Maize	20.0	
Rice	10.0	This diet is deficient in calcium, while containing a fat which is not strongly protective against rickets. There is a relatively large amount of phosphorus.
Rolled oats	10.0	
Peas	10.0	
Navy beans	10.0	
Casein	10.0	
NaCl	1.0	
$NaHCO_3$	1.5	
$CaCO_3$	0.2	
Butter fat	8.0	
	100.0	

Blood from heart:

R. B. C.	448,000
Hb.	less than 10%
W. B. C.	1,000
Reticulated R. B. C.	70%

The red cells showed marked anisocytosis and polychromasia and some poikilocytosis. A few nucleated red cells were seen. There was leucopenia with many early forms of the bone marrow series.

Autopsy. The tissues were pale. The liver was nearly white. The spleen was pale, but not enlarged. There were no gross signs of rickets.

B. A female rat, aged 24 days, weighed 18 grams. The mother had been on the following diet 60 days before the birth of her litter:

Rice	10.0	
Wheat	32.5	
Maize	15.0	
Peas	10.0	This diet is deficient in calcium and fat.
Navy beans	10.0	
Rolled oats	10.0	
Casein	10.0	
NaCl	1.0	
$NaHCO_3$	1.5	
	100.0	

Blood was taken from the heart. The blood was thin and watery, and enough to count could not be obtained. Smears were made which showed fairly well marked anisocytosis and poikilocytosis and polychromasia. Many nucleated red cells were seen. There was a leucopenia.

Autopsy. The tissues and viscera were very pale. The spleen was not enlarged.

C. A female rat, aged 16 days, weighed 10 grams. The mother had been on the above diet for 84 days before the birth of her litter. Blood from heart:

R. B. C.	5,044,000
Hb.	75%
W. B. C.	1,200
Reticulated R. B. C.	10%

The red cells showed slight polychromasia. There was a marked leucopenia, but the white cells seen appeared to be normal. No nucleated red cells were seen.

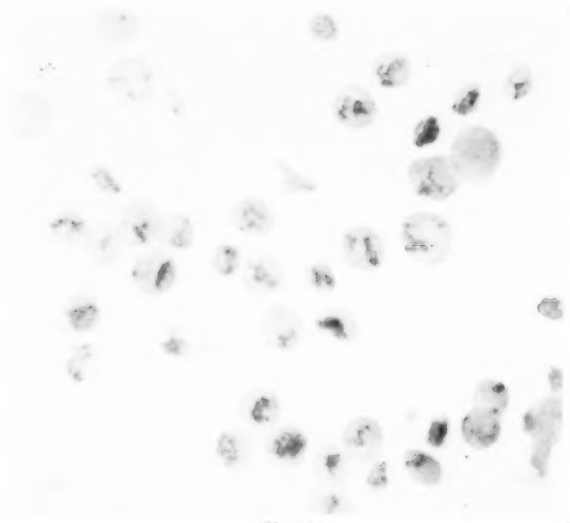

Fig. I.

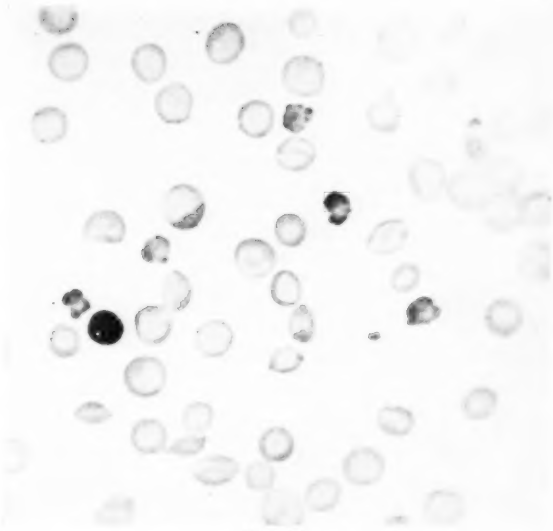

Fig. II.

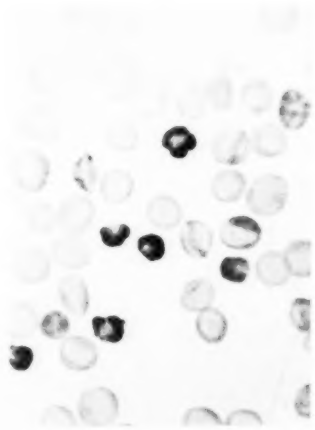

Fig. III.

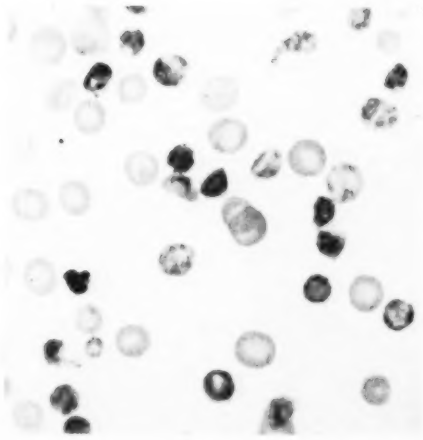

Fig. IV.

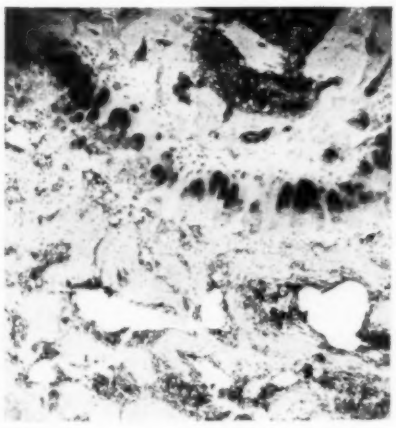

Fig. V.

Fig. VI.

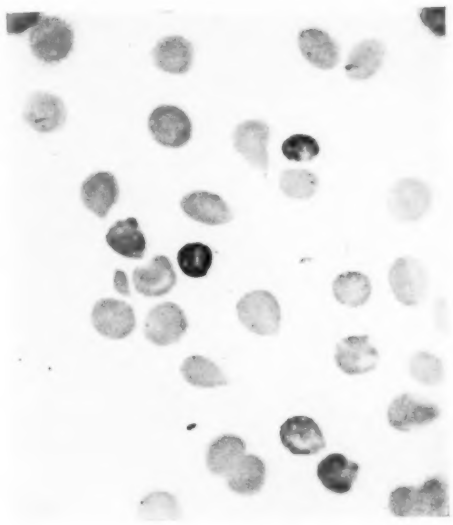

Fig. VII.

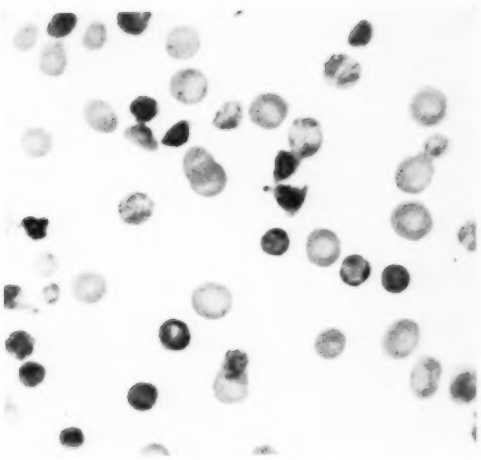

Fig. VIII.

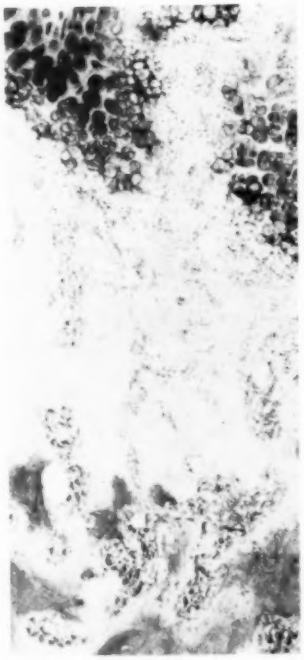

Fig. IX.

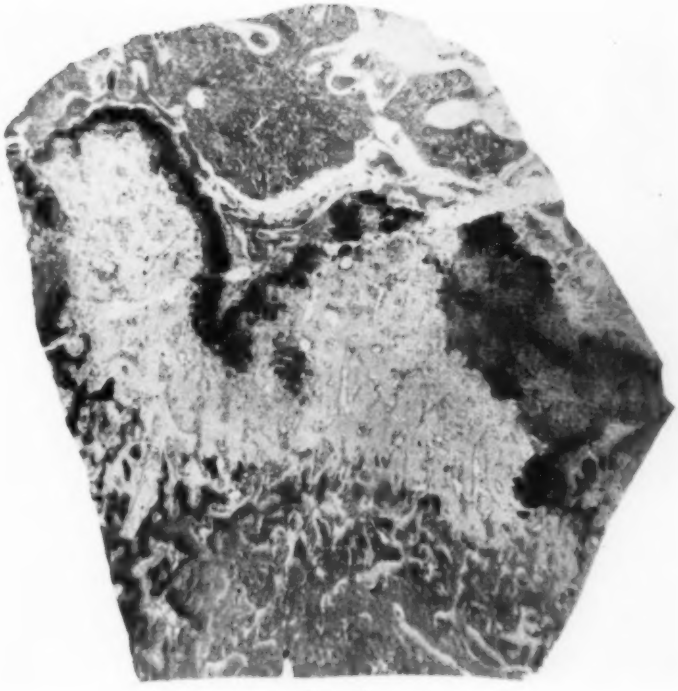

Fig. X.

Autopsy. The spleen was small. There was no thoracic deformity. The costo-chondral junctions were slightly enlarged. The long bones were thin and cut easily.

Microscopic sections showed well calcified osteoporotic bones.

D. A female rat, aged 19 days, weighed 14 grams. The mother of this animal had been on the following diet 70 days. She continued on the diet while she nursed her litter:

Wheat	30	
Maize	30	
Rice	10	This diet is deficient
Rolled oats	10	in calcium and fat.
Peas	10	
Navy beans	10	
	100	

Blood from thigh vein:

R. B. C.	6,208,000	P M. N.	15
Hb.	70%	Lymph.	80
W. B. C.	not counted	T.	5
			100

There was anisocytosis, poikilocytosis and a moderate degree of polychromasia. An occasional nucleated red cell was seen. The animal had a marked leucopenia.

The bones of the mother showed typical rickets. The bones of the second generation rat showed no osteoporosis and no rickets.

In five other rats of the second generation a practically normal blood picture was observed.

DISCUSSION

A diet deficient in iron alone does not produce anæmia in the rat in the first generation. It would appear either that the rat is born with a store of iron sufficient to carry him through life, or that only a very small quantity of iron is necessary to meet his requirements. On the other hand, diets deficient in an organic substance contained in cod-liver oil and to a less extent in butter fat and which also have certain improper calcium-phosphorus ratios (diets which produce rickets in the rat) produce anæmia in some animals. The anæmia in these animals is not due simply to a deficiency of iron in the diet. While these diets contained only a small amount of iron, they contained as much of this element as the stock diet or the other diets used upon which no anæmia developed. It would seem that the disturbance in the inorganic metabolism resulting from certain diets with which there is a lack of calcification of the bones also resulted in, or was accompanied by, a disturbance of the iron metabolism. It will be noted that the phosphorus content was relatively normal or increased and calcium relatively low in all the diets upon which anæmia developed.

When, however, the diet contained relatively excessive amounts of calcium but was low in phosphorus, no anæmia was produced in the rats which I examined. Diets of this type cause an exaggerated form of rickets to develop in rats and the calcium and phosphate content of their blood is identical with that seen in many severely rachitic children.

Milk diets except those in which casein was present in high percentage did not produce anæmia. Two rats which received the latter diet were the only animals in the series on diets of milk or modified milk which showed profound disturbance of calcification of the bones. I have no explanation to offer for this at present.

Enlargement of the spleen appeared to be as variable a feature in rats with rickets as it is in children with rickets. Enlargement of the spleen was frequently observed in rats in association with an anæmia of the actively regenerating type just as splenomegaly is commonly observed in infants with rickets and anæmia. However, although rickets makes its appearance relatively early in the rat, especially when the animal is put on the faulty diet at an early age, anæmia does not make its appearance until the rat has been on the diet for a long period. For this reason it is difficult to study anæmia produced by faulty food in rats, since they usually die from malnutrition or embarrassed pulmonary or cardiac function resulting from the marked thoracic deformity, or from pulmonary infection, before there is time for the anæmia to become manifest. The rats in which anæmia occurred had been on the faulty diet for a long period, or the anæmia was observed only in the second generation. Rachitic rats are usually sterile, so that second generations are not often raised.

The examination of films of the blood of the same rat, made simultaneously, the one stained with cresyl blue and Wilson's stain following the technique outlined above, the other with Wilson's stain alone, showed that if the polychromatophilic (diffusely basophilic) red blood cells and the reticulated erythrocytes were counted, the counts were practically the same. This would seem to indicate, at least as far as the rat is concerned, that polychromasia of the red blood cells is an indication of a young cell. Because of the stimulation of the bone marrow which occurs in the course of anæmia of the type described in these rats, these immature cells are poured into the circulation in large numbers. They compose a large percentage of the red blood cells present. In the blood of the normal adult rat they are seldom encountered.

Although the anæmic animals were not treated, it would seem logical to assume that these anæmias require anti-rachitic therapy primarily. Iron would not seem to be primarily indicated because the amount of iron in any well balanced diet is sufficient to meet an animal's needs.

CONCLUSIONS

1. The normal blood picture of the rat varies with age. Young rats have a relatively lower red blood cell count, white blood cell count, and hæmoglobin percentage, and a higher lymphocytic percentage, than the adult rat. Diet and the presence of respiratory infections are factors which may influence the blood picture.

2. Well balanced diets, deficient in iron, do not produce anæmia in the rat in the first generation, nor do diets consisting solely of cow's milk or milk and bread. Slight anæmia may occur in rats of the second generation on these diets.

3. Diets deficient in fat soluble *A* or water soluble *B*, although they cause severe nutritional disturbances, do not produce anæmia in the rat. Diets so deficient in water soluble *B* as to produce polyneuritis diminish leucopoietic activity and cause a severe leucopenia with a shift to the right in the Arneth formula.

4. Diets low in an organic substance contained especially in cod-liver oil with a low calcium but high phosphorus content, which produce rickets-like changes in the rat, may also produce anæmia, provided the animal is kept for a long period on the diet. Animals of the second generation on this diet may also become anæmic. This anæmia is associated with evidences of increased hæmatopoietic activity. There is often an enlargement of the spleen. This condition resembles the anæmias seen in association with rickets in human beings.

5. A diet low in the organic substance contained in cod-liver oil and low in phosphorus with a normal calcium content, a diet that produces severe rickets with great uniformity, does not produce anæmia.

LEGENDS FOR FIGURES.

Fig. I.—Blood of normal newly born rat, stained with cresyl blue and Wilson's modification of Romanowsky's stain, showing the high percentage of reticulated red blood cells present.

Figs. II and III.—Blood of rat D, (p. 171), stained with Wilson's modification of Romanowsky's stain, showing one nucleated red cell and polychromasia of many red cells.

Fig. IV.—Blood of same rat stained with cresyl blue and Wilson's modification of Romanowsky's stain, showing reticulation of the red cells.

Figs. V and VI.—Sections of femur of same rat at different magnifications, showing rickets.

Fig. VII.—Blood of rat A (p. 170), stained with Wilson's modification of Romanowsky's stain, showing polychromasia.

Fig. VIII.—Blood of same rat stained with cresyl blue and Wilson's modification of Romanowsky's stain, showing reticulation of the red cells.

Figs. IX and X.—Sections of femur of same rat at different magnifications, showing rickets.

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THE SIGNIFICANCE OF THE INFLUENZA BACILLI

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Two main problems exist in regard to the influenza bacillus:—(1) Is it the cause of epidemic influenza, and (2) if not, what relation does it have to the disease and what is its general significance? The first of these questions, as will be pointed out below, we regard as settled—in the negative. It is with the second that this communication mainly concerns itself.

Is the influenza bacillus the cause of epidemic influenza? During the great pandemic of 1889-1891 Pfeiffer[1] noticed in smears from the pharynx and sputum of patients ill with the disease the organism which now bears his name. Briefly stated, the facts brought out by Pfeiffer and his associates were as follows: The bacillus could be found with great regularity in the secretions from the respiratory tract in cases of influenza, it was a small, gram-negative, aërobic organism requiring hæmoglobin guished true and pseudo-influenza bacilli on the basis of

for its growth on artificial media. Pfeiffer at first distinmorphology, the latter being larger and tending to grow in long threads, but later he seems to have concluded that all "influenza bacilli" were identical. One wonders now if Pfeiffer's pseudo-influenza bacillus was not the organism at present known as bacillus X or hemolytic influenza bacillus[2]. Studied as it was towards the end of the pandemic without there being ample opportunity for confirmatory work, nevertheless the organism of Pfeiffer seems at the time to have been generally regarded as the cause of the disease. In the light of present knowledge about this group of bacilli, it seems certain that Pfeiffer was dealing with no specific organisms, nor have we more than the bare fact of their association with influenza cases to support the idea of their etiological rôle in the previous pandemic.

During the twenty-five years which elapsed between the last two great epidemics one came to think of the influenza bacillus in a rather different way. It became apparent that it was not constantly present in the interepidemic outbreaks of mild influenza-like disease or grippe. Davis,[3] for example, in the epidemic of 1908 found influenza bacilli in four of twenty-three cases, and Lord [4] in three of twenty cases, while Moody and Capps [5] (1916), in a careful study of thirty-one severe instances of grippe, found them only twice. On the other hand it appeared that influenza bacilli could be isolated from normal people,[6, 7] and from a variety of pathological processes. Scheller[8] summarizes the literature on the occurrence of these organisms as follows:— epidemic influenza, the entire respiratory tract from nose to alveoli, tonsils-inflamed or normal, sinuses, middle ear infections, pleurisy, arthritis, pyelitis, orchitis, epididymitis, cystitis and urethritis; pyo- and hydro-salpinx, cholecystitis, tuberculosis (secondary), bronchiectasis, meningitis, and as part of a mixed infection in diphtheria, measles, scarlet-fever and whooping-cough (?). Needless to say all of these observations were made on a basis of morphology, staining, and simple cultural characteristics without attempt at adequate biological classification. During a rather extensive experience with clinical respiratory bacteriology from 1912 to 1918, we encountered influenza bacilli frequently in all types of acute and chronic respiratory infection. One is especially impressed by the tendency of the group to associate themselves with other bacteria. For example, we have on several occasions in cases of frank lobar pneumonia isolated *B. influenzæ* in pure culture from sputum when the lung puncture yielded pneumococcus alone. Luetscher's [9] experience in the course of careful observations on a large series of respiratory infections was a similar one. In 603 cases he isolated influenza bacilli in 28.5%. Lord [10] reports findings of the same kind. Before the recent pandemic, therefore, one would have been inclined to regard the influenza bacillus as a rather ubiquitous organism in the diseased and normal respiratory tract, unfolding its activities especially on tissues damaged by other infections. One may add that at this time no observations of consequence in regard to the biological relationship of the group were on record save perhaps those of Wollstein[11] who found some difference between meningitic strains and those from the respiratory tract.

The appearance of the pandemic in 1918 immediately revived an intense and general interest in the influenza bacillus. We shall discuss the voluminous literature of the past three years only from the point of view of its bearing on the etiology of the disease. Three main arguments have been brought out by those who believe the influenza bacillus to be the cause of epidemic influenza,— (1) The frequent presence of the organism in cases of the disease; (2) The production of experimental influenza in animals and in man by inoculation with the bacillus; and (3) The development of immunity reactions.

That the influenza bacillus was present during the recent pandemic with great constancy both in uncomplicated cases of influenza and in those followed by pneumonia is established beyond dispute. The uniformity of this finding followed directly the introduction of suitable media for the cultivation of the organism, especially the chocolate medium of Williams, and the oleate hæmoglobin agar of Avery. One need only quote such reports as those of Williams,[12] of Keegan,[13] of Rappaport,[14] and of Stillman and Pritchett[15] who found influenza bacilli in from eighty to one-hundred per cent of the cases as illustrations of the usual experience. We have found no records of work done during the epidemic with proper media and facilities which do not agree with this result. It does not seem fair, however, to draw definite etiological conclusions from such observations. By an exactly similar line of reasoning the micrococcus catarrhalis or the streptococcus viridans might be said to be the cause of influenza. One must recall in this connection the well known example of the hog-cholera bacillus.

The transmission experiments must be closely scrutinized. Blake and Cecil[16] report the production in monkeys, by inoculation with virulent influenza bacilli of a disease which they regard as identical with human influenza. While they have undoubtedly produced a pulmonary infection with the organism, we are not at all impressed by its similarity to *uncomplicated* human influenza. This experimental disease is perhaps roughly comparable to the *post-influenzal* pneumonias in man. The same scepticism may be entertained in regard to the experiences of Cecil,[17] and of Park,[18] who inoculated human beings. Mild indispositions with catarrhal symptoms, such as followed some of the inoculations, are too dubious to be regarded definitely as true influenza.

The immunity reactions which have been reported by some observers indicate no more than a reaction to the influenza bacillus.[19, 20, 21, 22, 23, 24, 14] They have no definite bearing on its causal rôle in epidemic influenza.

In summary, then, recent studies have failed to bring satisfactory proof that the influenza bacillus is the cause of epidemic influenza.

On the other hand, certain observations seem to weigh strongly against the case for the Pfeiffer bacillus. As early as the epidemic in 1918 Bloomfield and Harrop,[25] on clinical grounds, felt that the influenza bacillus could not be the cause of the disease. It seemed to us inconceivable that an organism which was so ubiquitous and so versatile in its activities and, as a rule, so obviously a secondary invader, could suddenly produce a disease of fixed and definite characteristics and one so essentially different from the known influenzal lesions. This view, needless to say, was held in regard to the primary disease and not the secondary pneumonias. Furthermore, the incidence of the Pfeiffer bacillus was found to be practically as high in other acute diseases occurring at the time of the epidemic as in influenza itself. One may mention, for example the observations of Sellards and Sturm,[26] who found influenza bacilli in twenty-five of thirty-one cases of measles. Again, transmission experiments conducted under such ideal conditions as those of Rosenau[27] and of Leake[28] cannot be altogether disregarded, although it must be admitted that negative results are never conclusive. But most important are the serological studies of Park and his associates [29, 30] of Coca,[31] of Bell [32] and of Chesney.[33] These men all failed to find that various strains of influenza bacilli from epidemic cases were identical, when tested by biological methods such as agglutination. As first emphasized by Park,[34] one can hardly believe that the pandemic could have been caused by anything but a highly specialized strain of the causal organism. Finally, the recent studies of Gates and Olitzky,[35] although as yet unconfirmed by others, are most suggestive. The finding of a filter-passing organism in the secretions of epidemic cases, which reproduces rather constant lesions in animals, deserves most careful consideration.

On the whole, then, it seems to us that the influenza bacillus or rather the group of so-called influenza bacilli can no longer be regarded as the cause of the epidemic disease.

What then is the nature of the relationship of the influenza bacillus to epidemic influenza, and what in general is its significance? It is important at this point to emphasize the fact now clearly established that "influenza bacilli" really represent a great group of organisms and not a single fixed type. The literature on this question is adequately reviewed by Rivers,[36] and by Stillman and Bourn.[37] In brief it may be said that while the organisms in question have certain common characteristics—to wit, that they are all small Gram-negative bacilli, hæmoglobinophilic on the ordinary laboratory media, [38] when classified by biological methods such as agglutination as well as by simpler criteria such as the production of hemolysis, indol formation, reduction of nitrates, amylase formation, and sugar fermentations, they fall into a large variety of sub-groups, the extent of which is as yet unknown.

None the less it is certain that as a whole influenza bacilli were tremendously prevalent at the time of and following the epidemic of 1918 both in patients with influenza and in other individuals. A comparison of the incidence before and after the epidemic can unfortunately not be made, inasmuch as the inadequate cultural methods previously used would hardly allow a fair estimate. It seems to us, however, that light may be shed on the question of the significance of the group by studying the occurrence of Pfeiffer bacilli now—three years after the pandemic—at a time when influenza once more seems extinct.

Briefly stated, then, the idea of the present study was as follows:— Even if one makes allowance for the inadequacy of bacteriological methods before the epidemic of 1918, it seems certain that at that time there was no widespread existence of influenza bacilli in normal healthy people. Davis,[6] in 1907, found them in two of twenty normal throats, and Holt [7] (1910) reports their presence in nineteen per cent of two hundred and fifty-four persons without respiratory disease. On the other hand, their incidence in pulmonary lesions as secondary invaders was high.[9, 10] But at the time of the epidemic their prevalence became wide, not only in cases of influenza but in normal individuals as well. Lord[39] in the midst of the pandemic found influenza bacilli in seventy-six per cent of thirty-four normal students, Pritchett and Stillman [15] in forty-two per cent of controls, Opie [40] in thirty-five per cent, and Jordan [41] in forty per cent. Even during the first two years after the epidemic the incidence in normals remained high—Winchell and Stillman [42] (39 per cent,) Pilot and Pearlman [43] (40 per cent) and Bloomfield [44] (90 per cent). It seemed of interest, therefore, to determine the occurrence of Pfeiffer bacilli now (1921-22) three years after the epidemic. Should it turn out that the incidence had dropped to a figure comparable to the pre-epidemic results, one would be forced to conclude that the presence of influenza alters the respiratory mucous membrane as a soil in such a way that *B. influenzæ* becomes temporarily adapted to a widespread saprophytic growth. Such a fact would be of interest not only on its own account but because of its general bearings on questions of bacterial adaptation.

In summary we have studied the following questions:—

1. The comparative incidence in the population at large during epidemic, post-epidemic, and later periods.
2. The seasonal variation, if any, through the year in relation to respiratory disease.
3. The relative number of chronic and of temporary carriers.
4. The localization of organisms in carriers in relation to persistence.

METHODS

A heterogeneous group of people was studied. It consisted of physicians, technicians, students, and patients. All were free from clinical respiratory disease. In making the routine cultures a cotton swab was passed over the pillars, tonsils, and pharynx as high as one could reach. The swabs were plated on Avery's oleate hæmoglobin agar [45] (pH 7.4-7.6), a technique previously described [46] being employed, which yields a discrete spread of colonies so that accurate counts can be made. Each batch of medium was tested before use by inoculation with stock strains of influenza bacilli. Unless luxuriant growth was obtained the medium was discarded. We regard this test as very important, since successive batches of Avery medium, even if prepared in the same way, may not be equally satisfactory. Suspicious colonies were identified by staining and by subculture on plain agar and on rabbit blood-agar.

TABLE I.

Incidence of Influenza Bacilli in Throats of Healthy People.

Before Pandemic of 1918	During Pandemic	During two years after Pandemic	Present Incidence
Davis 10% Holt 19%	Lord 76% Pritchett & Stillman 42% Opie 35% Jordan 40%	Winchell & Stillman 39% Pilot & Pearlman 40% Bloomfield 90%	Total cultures 283 Number positive 49
Average— not over 20%	Average — about 50%	Average — about 50%	Percentage positive 17.3%

TABLE II.

Comparative Incidence of Influenza Bacilli in Two Similar Groups of People.*

1920

Name		M	C	B	V	A	Ca	Bv	Ma	E	G	D
Culture	1	0	200	Few	∞	Few	200	∞	Few	0	0	0
	2	Few	300	100	Many	200	300	∞	Many	0	0	0
	3	∞	Few	Many	100	∞	Few	∞	Few	0	0	0
	4	Many	Few	Many	Many	Many	0	Many	Many	100		Many
	5	0	Few	Many	50		Few		Many	Few		Few
	6	Many	100	Many			Many			0		0
	7									0		0
	8									Many		Few
	9									Many		

* The numbers indicate the number of colonies recovered. ∞ —Innumerable.

1921-22

Name		H	R	C	B	L	M	Ho	A	Hu	Re	Ha
Culture	1	0	∞	0	0	0	0	0	0	0	0	0
	2	0	∞	0	0	0	0	0	0	0	0	50
	3	0	Many	0	∞	0	0	0	0	0	0	0
	4	0	90	0	100	0	0	0	0	0	∞	0
	5	0	2	0	100	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0
	7	0	6	0	0	0	0	0	0	0	0	0
	8	0	100		0	0	0			0	0	0
	9	0	20		0		0				0	
	10	0	∞		0		0				0	
	11		Many									

RESULTS

1. *The Comparative Incidence of Influenza Bacilli at Various Times.* It was our aim in this part of the study to make single cultures in a large group of healthy individuals free from respiratory disease in order to determine the incidence of the influenza bacillus now, in comparison with its occurrence before, during, and after the pandemic. During the months of August to December 1921 a miscellaneous group of two hundred and eighty-three people was examined. Of these, forty-nine, or 17.3%, yielded Pfeiffer bacilli in numbers varying from a few colonies up to almost pure plates. Table I shows figures derived from the literature in comparison with our present results. It is seen that the increase which occurred at the time of the epidemic persisted for about two years, but that now (1921-22) the incidence is much lower,—in fact quite comparable to that which existed prior to the pandemic. This point can be brought out still more clearly (Table II) by comparing in detail two sets of cultures of our own, the first made during the spring of 1920, the second during the winter of 1921-22. Exactly the same methods were used and the groups of people were similar, consisting of healthy physicians and technicians working in the laboratory. Cultures were taken at intervals of from one to three weeks. It is seen that in the first series all but one individual harbored influenza bacilli almost constantly, whereas in the second group there was only one constant and three transient carriers.

In summary, then, it appears that the high incidence of influenza bacilli in the throats of healthy people which was present during and after the pandemic has declined to the "normal" or pre-epidemic figure.

2. *The Seasonal Variation and the Relation to the Incidence of Respiratory Disease.* In each subject studied a careful examination of the throat was made for evidences of abnormality such as diseased tonsils. The history of previous nasal or throat infection or of respiratory disease was also investigated. In brief it may be said that absolutely no relationship between the presence of influenza bacilli, and the occurrence of previous upper air passage infection could be demonstrated in the present observations. It seemed important, also, to determine the seasonal variation, if any existed, in the incidence of influenza bacilli in normal people. Table III shows our cases arranged according to months from August to December. No significant variation appears, although the percentage of positive cultures was somewhat greater during October, November and December than it was in August and September. These results are in accord with those of Winchell and Stillman who found a fairly steady incidence from December 1918 to April 1919, although their figures were much higher than ours.

In summary, then, there is no convincing evidence that the incidence of influenza bacilli in healthy people varies with the incidence of mild respiratory infection.

TABLE III.

Seasonal Incidence of Bacilli in Healthy People

		Positive	Negative	Per cent Positive
August	1921	8	46	14.8
September	1921	5	40	11.1
October	1921	16	54	23.0
November	1921	10	42	19.2
December	1921	10	52	16.0

3. *Carriers.* In the present studies our interest in influenza bacillus carriers has centered in the question whether any law governing the adaptation of these organisms to growth on human mucous membranes could be deduced. The literature considered from this point of view is meager.

(a) *Carriers Associated with Chronic Disease.* As pointed out above, the influenza bacillus is often found in chronic pulmonary lesions. Lord [10] and Luetscher [9] report its presence in a variety of conditions; Boggs [47] pointed out its frequency in bronchiectasis; and Miller [48] has stressed its presence in chronic basilar non-tuberculous infections.

(b) *Carrier States Following Experimental Inoculation of Influenza Bacilli in Man.* Wahl, White and Lyall [49] applied a saline emulsion of influenza bacilli from epidemic cases to the mucous membrane of the nares and pharynx of five healthy men. They were unable to recover the organisms from the nose after forty-eight hours except in one case, but found them present in the naso-pharynx for two weeks or longer. In some cases the bacilli disappeared after a few days to reappear later. There is doubt, therefore, as to the identity of the strains recovered on successive cultures. Sellards and Sturm,[26] following inoculations into the nose and throat, recovered no influenza bacilli after three days. Bloomfield [50] used three strains isolated from healthy men. In twelve experiments in which inoculations were made on various areas of the upper air passages the strain introduced could not be recovered after forty-eight hours, but in five cases Pfeiffer bacilli isolated later than twenty-four hours were found to differ from the experimental strains. Davis [51] found influenza bacilli in the throat of a volunteer four weeks after inoculation. Cecil and Steffen,[17] after experimental inoculations with supposedly virulent strains, noted a persistence of bacilli lasting from six hours to two months in various cases. The identity of the strains was checked by agglutination tests. Park and Cooper [18] studied four cases of accidental inoculation with influenza bacilli. In one instance no carrier state was set up, in the others bacilli were present after 4, 28, and 68 days respectively.

(c) *Spontaneous Carrier States in Normal (Clinically Healthy) People.* Povitzky and Denny [30] found that influenza bacilli persisted in the throat for lengths of time up to one year. Serological tests showed that the strain changed in some cases, while in others the same organism persisted. Bloomfield [44] found influenza bacilli

repeatedly during a period of about three months in nine individuals. No proof of the identity of strains from a given carrier was obtained. Winchell and Stillman [42] made careful studies on a group of people during the winter of 1918-19. Cultures were made every month on the same eighty-five individuals. Fifteen never yielded a positive result, nine of thirty-five positive on the first examination never showed influenza bacilli later, six of the eighty-five were positive for five months and thirteen for four months. Unfortunately, it was not determined whether the same strains persisted or whether new ones appeared from time to time.

In *summary*, then, the literature brings out the following points in regard to carriers of influenza bacilli.

(1) Influenza bacilli may be carried for prolonged and indefinite periods of time by individuals affected with chronic respiratory disease—bronchitis, bronchiectasis, lung abscess, etc. In this case the organisms seem to live and multiply in the focus of infection whence they are discharged.

(2) Normal (clinically well) people without any recent history of respiratory disease may harbor influenza bacilli in the throat. While the observations on record are few, it appears that such a carrier state may be brief or prolonged, and that in some cases the same strain of organism may be present for months or a year.

(3) Great variations have followed the experimental introduction of influenza bacilli in man. In some cases the organisms were promply eliminated, in others a carrier state was set up lasting for weeks or even months. The situation is complicated by the doubtful relation of some of the cases to acute respiratory infection, by the fact that no exact observations were made on the location of the organisms in the upper air passages, and by the fact that in some cases old laboratory strains and in others freshly isolated human strains were used.

Furthermore, since the observations in normals were made during or shortly following the epidemic, at a time when the behavior of influenza bacilli was a variable affair, no conclusion can be drawn from them as to the normal adaptive power of *B. influenzæ* to human mucous membranes.

TABLE IV.

Results of Serial Cultures in Subject B.

The subject is a physician frequently exposed to respiratory infection. No recent history of colds or sore throat. Throat clear. Small clean tonsils.

Culture Aug. 19, 1921	No influenza bacilli	
Culture Aug. 29, 1921	No influenza bacilli	For past 24 hrs. slight pharyngitis with malaise
Culture Sept. 7, 1921	No influenza bacilli	Well
Culture Sept. 16, 1921	Innumerable influenza bacilli	No recent infection
Culture Sept. 17, 1921	About 100 cols. influenza bacilli	
Culture Sept. 20, 1921	About 100 cols. influenza bacilli	
Culture Sept. 30, 1921	No influenza bacilli	
Culture Oct. 4, 1921	No influenza bacilli	
Culture Oct. 17, 1921	No influenza bacilli	
Culture Nov. 9, 1921	No influenza bacilli	
Culture Dec. 12, 1921	No influenza bacilli	

Comment.—It is seen that B. developed a transient carrier state lasting only a few days and apparently quite unrelated to any external factor.

TABLE V.

Results of Serial Cultures in Subject H.

The subject is a physician frequently exposed to respiratory infection. No recent history of cold or sore throat, but he has very large scarred tonsils with plugged crypts.

Culture Oct. 2, 1921	No influenza bacilli	Has been in contact with a patient with chronic bronchitis and a pure culture of the influenza bacillus in sputum
Culture Oct. 4, 1921	50 cols. influenza bacilli	
Culture Oct. 5, 1921	No influenza bacilli	
Culture Oct. 17, 1921	No influenza bacilli	
Culture Oct. 30, 1921	No influenza bacilli	
Culture Nov. 9, 1921	No influenza bacilli	
Culture Nov. 15, 1921	No influenza bacilli	
Culture Dec. 12, 1921	No influenza bacilli	

Comment.—An example of a very transient carrier state, the source of the influenza bacilli was possibly the patient referred to above. Two cultures were made from material aspirated from the crypts of H's tonsils. No influenza bacilli were recovered.

TABLE VI.

Results of Serial Cultures in Subject R.

The subject is a physician exposed to respiratory disease. Not subject to frequent sore throats. Very small clean tonsils.

Culture on Sept. 18	No influenza bacilli	
Culture on Sept. 30	No influenza bacilli	Slight pharyngitis—mild constitutional symptoms. Throat slightly red
Culture on Oct. 3	No influenza bacilli	Feels well. Throat clear
Culture on Oct. 17	Innumerable influenza bacilli	Sore throat for 1 day. Throat looks red, patches on tonsils Outspoken follicular tonsillitis Temp. 101.°
Culture on Oct. 19	4 cols. influenza bacilli	Blood-agar plates show almost pure culture of hemolytic streptococcus.
Culture on Oct. 21	6 cols. influenza bacilli	Throat better. Still many hemolytic streptococci
Culture on Oct. 26	No influenza bacilli	Well
Culture on Nov. 9	No influenza bacilli	
Culture on Nov. 21	No influenza bacilli	
Culture on Dec. 12	No influenza bacilli	

Comment.—It is seen that a transient carrier state was present. With the coincident onset of a streptococcus tonsillitis the influenza bacilli rapidly disappeared. Culture from the tonsil crypt showed no influenza bacilli.

TABLE VII.

Duration of Carrier State from Time of First Positive Culture.

Duration	*Number of Cases*	*Remarks*
5 months	1	Still positive on last culture
3 months	1	
1 month	1	Still positive on last culture
2 weeks	1	Still positive on last culture
10 days	4	1 case still positive on last culture
7 days	4	1 case still positive on last culture
4 days	2	
2 days	3	
1 day	1	

Our own observations were conducted along two lines. First a set of ten available people was selected. They were all normal clinically and were free from respiratory disease. Cultures were made from time to time over a period of four to five months to see if any members of the group would become carriers and, if so, what the course of the carrier state would be. Cultures from all ten men were negative at first. Seven failed to show influenza bacilli at any time. The other three developed temporary carrier states which were of interest. Their records are presented in Tables IV, V and VI. It is seen that about one third of the group developed a transient infestation with influenza bacilli, emphasizing the probability of an extensive give and take of these organisms among the population at large, independent of respiratory infection and without permanent colonization.

Next a study was made of the persistence and localization of *B. influenzae* in a larger group of carriers. Of the forty-nine subjects who showed positive cultures eighteen were available for further observations. The duration of the carrier state in these people is shown in Table VII. The striking point is the generally brief duration of the carrier state, which lasted ten days or less in twelve of the eighteen cases.

It is a well known fact that carrier states discovered in healthy individuals are usually very persistent. Diphtheria bacilli, as pointed out especially by Guthrie, Moss, and associates,[52] when once present, usually persist over long periods of time. The same is true of hemolytic streptococcus and Friedländer carriers.[53] Furthermore, in these cases it can usually be shown that the foreign organism is harbored in a local focus of diseased tissue such as the tonsil. It seemed of importance, therefore, to determine in the case of influenza bacillus carriers whether any relationship existed between the duration of the carrier state and the localization of the organisms in the throat. A series of differential cultures was therefore made the results of which are shown in Tables VIII–XIII.

TABLE VIII.

Differential Cultures from Carrier R. This subject is a healthy man. He says that his tonsils have never been removed, but little tonsillar tissue is visible. The throat is clear.

	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
No. cols. B. influ.	Aug. 18, 1921	∞	∞	∞	0
	Aug. 29, 1921	many	many	many	0
	Sept. 19, 1921	0	40	50	0
	Sept. 30, 1921	0	0	0	0
	Oct. 4, 1921	0	6	0	0
	Oct. 19, 1921	100	2	2	0
	Nov. 4, 1921	0	0	20	0
	Nov. 11, 1921	0	∞	∞	0
	Dec. 14, 1921	many	many	a few	0

Comment.—No tests were made to prove the identity of influenza bacilli recovered on successive cultures. It is seen, however, that there was no sharp localization of the organisms at any special site.

TABLE IX.

Differential cultures from Carrier V. Healthy man. Huge globular tonsils almost meeting in midline. No acute process at present.

	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
No. cols. B. influ.	Nov. 7, 1921	300	0	a few	0
	Nov. 8, 1921	200	200	20	0
	Nov. 9, 1921	∞	100	∞	0
	Nov. 11, 1921	∞	100	35	1

Comment.—No localization made out.

TABLE X.

Differential Cultures in Carrier G. Patient has acute nephritis. Tonsils removed two weeks ago. Still a small raw area on the right, but throat is clear.

	Site of Culture	Rt. Tonsil (fossa)	Lt. Tonsil (fossa)	Pharynx	Soft Pal.	Tongue
No. cols. B. influ.	Nov. 5	0	0	∞	0	0
	Nov. 7	0	0	150	0	0
	Nov. 9	0	0	100	0	0
	Nov. 11	0	0	0	0	0
	Nov. 14	0	0	20	0	0
	Nov. 18	0	0	0	0	0
	Nov. 28	0	0	0	0	0

Comment.—Although the carrier state was of relatively brief duration, the organisms were confined to the pharyngeal wall.

TABLE XI.

Differential cultures in Carrier H. Multiple neuritis. Not subject to sore throats. No recent respiratory infection. Very small clean tonsils. Nose and throat examination by specialist, negative.

	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
No. cols. B. influ.	Nov. 5	0	0	∞	0
	Nov. 7	0	0	200	0
	Nov. 9	0	0	0	0
	Nov. 11	0	0	50	0
	Nov. 14	0	0	∞	0
	Nov. 18	0	0	0	0
	Dec. 8	0	0	0	0
	Dec. 12	0	0	0	0

Comment.—A brief carrier state with the organisms confined apparently to the pharynx.

TABLE XII.

Differential cultures in Carrier Gr. Normal man. Rather large globular but clean tonsils. Not subject to respiratory infections.

	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
No. cols. B. influ.	Aug. 22	many	many	many	0
	Aug. 25	40	0	2	0
	Sept. 5	0	0	0	0
	Sept. 12	0	0	0	0
	Sept. 17	0	0	0	0

TABLE XIII.

Differential cultures in Carrier N. Healthy man. Small ragged tonsils. Not subject to sore throats. No recent respiratory infection.

	Site of Culture	Rt. Tonsil	Lt. Tonsil	Pharynx	Tongue
No. cols. B. influ.	Aug. 20	200	300	0	0
	Aug. 26	150	6	0	0
	Sept. 7	200	∞	0	0
	Oct. 19	many	many	many	0
	Nov. 20	0	0	0	0
	Nov. 26	0	0	0	0

It is seen that in the case of carrier states of varying lengths no definite law of localization can be derived. The organisms may be confined to one or another area or may vary from time to time in their distribution. A check on the above findings was provided by making a series of twelve cultures from material aspirated from the depths of tonsil crypts. In two cases a few influenza bacilli were found. These findings are therefore in contrast to the conditions obtaining in carriers of streptococci (hemolytic), diphtheria bacilli, Friedländer bacilli and others, where one usually deals with a persistent localization in the depths of infected lymphoid tissue.

DISCUSSION

The main results of the present work may be briefly summarized as follows. In the first place it appears that the general incidence of influenza bacilli in the throats of healthy people has declined greatly since the time of the pandemic, the present figure being comparable to that which existed before the wave of influenza in 1918. Furthermore, there seems to be no striking relationship to the season of the year or to the prevalence of minor respiratory infections. An outstanding feature of the carrier state in the individuals we have observed was its usual relatively brief duration and the absence of evidence in the case of healthy people of localization of influenza bacilli in a localized area of diseased tissue such as the tonsil.

A few words may now be said in regard to the general bearings of our findings on the broader question of bacterial adaptation to growth on respiratory mucous membranes. In another place[54] it was pointed out that criteria of test-tube growth do not serve to explain the presence in the throat of certain organisms and the absence of others, but that one must assume subtle biological adaptations, the exact chemistry of which is as yet obscure. It was shown that bacteria such as green-producing streptococci possess a high degree of inherent adaptation, as indicated by their early and constant appearance in the throats of infants and their almost constant presence in adults as well. On the other hand, other organisms such as *B. coli* seem to have a total lack of adaptation to growth in these regions. Furthermore, evidence was presented that still other organisms possess partial degrees of adaptation,[55] occurring frequently but not constantly. The hemolytic influenza bacilli, for example,—which as far as we know play a negligible part in human pathology—are found in from 30 to 50 per cent of throats of healthy people, as a rule unassociated with any focal infection but seemingly growing free on the mucous membranes. The question which now logically arises is whether the adaptation of organisms to growth on mucous membranes may not undergo waves of fluctuation depending on a variety of factors. Many observations in the literature give hints that this may be so. Hemolytic streptococci, while frequently present in focal infections in diseased tonsils,[56] are not apt to be found on the free surfaces of the normal mucous membranes.[57] When experimentally introduced into the throat, they either disappear[58] rapidly or set up a tonsillitis in association with which they can be recovered in culture.[17,49] It is clear, then, that the present average adaptation of hemolytic streptococci to free growth on normal mucous membranes is very low and that a local infection is necessary for permanent colonization. During the war, however, at a time when malignant streptococcus disease was epidemic, the passage of hemolytic streptococci through many people seems to have altered the organisms in such a way that a much higher adaptation to growth on mucous membranes existed. Hence these organisms were found in a high percentage of throat cultures from normal people[59] in contact with streptococcus disease. Even here, however, the carrier state seems to have been temporary, and after the epidemic it promptly reverted to the average conditions mentioned above. One may cite also in this connection the frequent association of hemolytic streptococci with scarlet fever and other acute infections. Similar considerations seem to apply to the meningococci. In interepidemic times they are practically never found in healthy people and then probably only in local foci of infection, whereas during epidemics the carrier rate may be as high as twelve to eighteen per cent.[60] Clearly an alteration has temporarily taken place which leads to a greater degree of adaptation to growth on mucous membranes.

The influenza bacilli, however, present by far the most picturesque example of such changes in adaptation. The normal state of affairs in regard to the adaptation of these organisms seems to be that they show a widespread association with chronic focal infections in the respiratory tract. There is no evidence that they have more than a moderate and partial degree of adaptation to free growth on normal mucous membranes. A variety of acute infections, however, alter the soil in such a way that influenza bacilli flare up as it were and temporarily become adapted to widespread growth in the affected individuals. This has been shown to be the case in scarlet fever (39%) by Jehle,[61] in diphtheria (60%) by Jehle, in varicella (100%) by Jehle, and in measles (78%) by Jehle, (47%) by Susswein, (33%) by Wollstein, (56%) by Davis and (79%) by Sellards. The observations of Sellards[26] are of particular importance, because exami-

nations were made at various stages of the disease. Influenza bacilli were found in seventy-nine per cent of the cases during the eruptive stage, in twenty-four per cent ten to fourteen days after eruption, in twenty per cent twenty to twenty-five days after eruption, and in no case forty days after the eruption. In these diseases, therefore, the adaptation is temporary and apparently not of a degree high enough to allow growth to extend generally to the mucous membranes of healthy people.

It is of interest that the mild epidemic grippe and the common cold are relatively inadequate to produce such marked alterations in adaptation.[62] Epidemic influenza, on the other hand, produces this effect to a maximal degree. The result was, as has been pointed out, not only an almost uniform incidence in cases of the disease but in the normal population as well. This change persisted for at least two years after the epidemic, but now, three years after the epidemic, conditions, as indicated by our figures, seem to have returned to the normal average. In summary, then, we have in the case of the influenza bacillus group, organisms which show a marked variation at various times in their adaptation to growth on human mucous membranes. A variety of altered conditions, especially acute infectious diseases, may produce this change but epidemic influenza does so to an extreme degree.

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THE INTERCOLUMNAR TUBERCLE, AN UNDESCRIBED AREA IN THE ANTERIOR WALL OF THE THIRD VENTRICLE

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In a recent paper,[1] it was pointed out that the areæ postremæ of the fourth ventricle became deeply colored in animals injected with trypan blue. It was supposed that this structure was the only intrinsic portion of the brain which took such a stain. A more minute examination of the brains of some of these animals, however, revealed the presence of a small tubercle of tissue, similar to that composing the areæ postremæ, in the anterior wall of the third ventricle. But while the areæ postremæ were probably known to Stilling, and were well described by Retzius, the analogous area in the third ventricle has apparently been overlooked by anatomists.

This area is situated between the anterior pillars of the fornix, about on a level with the superior border of the foramina of Monro in the species studied, and below the juncture of the two choroid plexuses of the lateral ventricles. It might therefore appropriately be called the *intercolumnar tubercle*. It may be exposed, in the animals observed, by an accurate frontal section passing just in front of the optic chiasm. In the fresh unstained specimen, it can usually be recognized without difficulty as a pearly, grayish, translucent nodule, round or somewhat fusiform and of a slightly variable size, up to one millimeter in diameter and half a millimeter in height. In animals stained with trypan blue or other colloidal dyes (pyrrhol blue) there is some individual variation in depth of staining, but the tubercle is almost always distinctly colored. It may also be brought out by a capillary injection of blood-vessels, or by staining the gross specimen with dilute methylene blue, or certain other diffusible dyes (Bismarck brown, neutral red), when the tubercle takes a distinctly brighter color than the surrounding tissue. In unstained tissues fixed by the ordinary methods, its peculiar pearly appearance is lost, and it can be identified only by its shape and position. It is frequently reduced in size also by fixation. In the human brain, the choroid plexuses must be lifted up or stripped away before it can be seen. It varies but little in size, shape, and position in the various animals studied (rat, guinea-pig, rabbit, cat, dog, monkey and man).

Histologically, the intercolumnar tubercle resembles the area postrema. It is mainly composed of a very loose reticulum of neuroglia cells, which, however, differs markedly in appearance from the fine, regular felt-work seen elsewhere in the brain. The nuclei of the cells composing it are mostly large and elongated, measuring up to 20x10 micra, although smaller and round ones are seen. They stain less deeply with basic dyes than the ordinary spider-cells, and contain a distinct nucleolus and numerous coarse chromatin granules. The cytoplasm is indefinite in outline, but usually extends two or three micra beyond the nucleus and gives off several short processes. Occasionally it contains large vacuoles. It takes a faintly basic stain. Through it pass wavy fibrils, running from one cell to another; but they are much coarser and longer than the ordinary neuroglia fibrils, less numerous and more widely spaced. Thus, the tissue resembles a mesodermal reticulum in structure more than it does neuroglia, but none of the fibers are stained by picrocarmine or anilin blue, while nucleus, cytoplasm and fibrils are stained blue by Mallory's phosphotungstic hematoxylin. In addition, the relation of the tissue to the ependyma and to the remainder of the brain speaks against a possible mesodermal origin.

In the human brain, the tubercle contains large, triangular cells which resemble nerve-cells. The nucleus is pale, round or oval, about 20 micra in diameter, and has a large, dark, eccentric nucleolus and a few coarse chromatin granules. The cytoplasm takes a faint basic stain, and can be seen to be slightly granular. They are similar to the nerve-cells described by Wilson[2] in the area postrema.

In the monkey (Macacus rhesus) rather smaller cells, still somewhat resembling nerve-cells, are seen in the intercolumnar tubercle and (contrary to a previous report[1]) also in the area postrema. They have not been demonstrated in either situation in the lower quadrupeds.

The ependyma over the intercolumnar tubercle is squamous or very low cuboidal, resembling almost a mesothelial lining. It blends gradually with the columnar ependyma over the fornix. It is extremely delicate, and is often injured by even gentle handling.

The tubercle contains many coarse capillaries, with abundant perivascular connective tissue, in which are found the particles of dye in vitally stained animals. The adventitia can also be strikingly demonstrated by Van Gieson's or Mallory's connective-tissue stains. Injections show that these capillaries form a close network, supplied by one of the central branches of the anterior cerebral arteries, and draining into the internal cerebral veins. They do not communicate directly with the choroid plexus, although so close to it. This vascular arrangement is quite analogous to that of the areæ postremæ.[1]

The constancy of this structure, its vascularity, its peculiar neuroglia and ependyma, its behavior toward

vital dyes, and (in the human brain) the presence of nerve-cells, all suggest that it has some specialized function. But what this may be, we can at present barely speculate.

It is a pleasure to acknowledge my indebtedness to Dr. George Wislocki for many fruitful suggestions; to Professor Meyer for valuable criticisms; to Dr. George W. Corner for the monkey's brain; to Professor Brödel for assistance with the drawings; and to Professor W. MacCallum for permission to work on this problem, and for help and advice.

SUMMARY

A structure in the anterior wall of the third ventricle of the mammalian brain is described, which appears to be analogous in many ways with the well-known areæ postremæ of the fourth ventricle. It is a small prominence, up to one millimeter in diameter, situated between the columns of the fornix, at the level of the upper border of the foramina of Monro, below the juncture of the two lateral choroid plexuses. It is composed of a peculiar loose neuroglia tissue, containing many coarse capillaries, and covered by low ependyma. In the human brain, it contains small nerve cells. The adventitia of the capillaries takes up trypan blue and other vital dyes *intra vitam*. No function can at present be assigned to it. The name intercolumnar tubercle is suggested for this structure.

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DESCRIPTION OF FIGURES

1. Brain of human baby at term. (X1.) Slightly oblique section passing through the anterior commissure posterior to the juncture of the lateral choroid plexuses, and anterior to the optic chiasm. The enclosed area is seen slightly enlarged in the photograph (Fig. 2).

a—intercolumnar tubercle.
a—anterior columns of the fornix.
c—choroid plexus.
d—tip of temporal lobe.

2. Detail of enclosed area in Fig. 1. Slightly enlarged. Unretouched photograph.

3. Human brain. Sagittal section through intercolumnar tubercle, low-power photomicrograph. Formaldehyde fixation. Phosphotungstic acid hematoxylin. The intercolumnar tubercle is seen as a low eminence just to the right of the attachment of the choroid plexus, marked by its loose reticular structure and the flatness of its ependyma. The wide capillaries are surrounded by a generous amount of adventitia. A few nerve-cells are seen. The enclosed area is enlarged in Fig. 4.

4. Detail of enclosed area in Fig. 3. High-power camera lucida drawing. The characteristic flat ependyma is seen at the left, blending with the usual columnar ependyma at the right of the picture. The looseness of the mesh-work, and the size and shape of the neuroglia cells is shown. The shrunken adventitia of a large capillary is seen near the lower left-hand corner, and a smaller one in the right corner. Several nerve-cells are illustrated.

5. Brain of dog. Slightly enlarged. Blood vessels injected with india ink, cleared in oil of wintergreen. Frontal section passing through optic chiasm. Lens enlargement. The area enclosed by lines, containing the intercolumnar tubercle, is enlarged in Fig. 6.

6. Detail of enclosed area in Fig. 5. The intercolumnar tubercle appears as a small vascular mound near the center of the drawing.

PSEUDOMYXOMA PERITONEI

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INTRODUCTORY

In pseudomyxoma peritonei all or a part of the peritoneal surface is covered, and the body cavity, locally or generally, filled with gelatinous material, while the peritoneum itself usually shows some roughening and thickening. The jelly-like substance may occupy only part of the pelvis or one iliac fossa; or, in the extreme cases, the abdomen may be greatly distended by the semi-fluid material which fills practically all available space within the peritoneum. Sometimes the peritoneum is only moderately injected, at other times it may be enormously thickened, in some cases almost to a centimeter. Whitish bodies, pin-head to pea-sized, may be found, either as excrescences from the peritoneum or free in the gelatinous secretion; in these bodies there may be glandular masses, apparently of the nature of metastases.

HISTORICAL

The first important modern literature on the subject of pseudomyxoma peritonei dates back to 1884, when Werth described the condition and declared that it always has its origin in the so-called pseudomyxomatous tumors of the ovary. This author averred, moreover, that the peritoneum itself shows only a foreign-body reaction. Olshausen and Pfannenstiel, however, a little later predicated actual peritoneal metastases or implantations, saying that the extruded gelatinous material includes epithelial cells which continue to multiply and produce more of the same material. Wendeler, in 1896, follows Werth and speaks of a chronic productive or adhesive peritonitis, resulting from the rupture of a gelatinous cyst of the ovary. Fränkel, in 1901, made a notable contribution, reviewing the entire subject, and then describing two cases of his own.

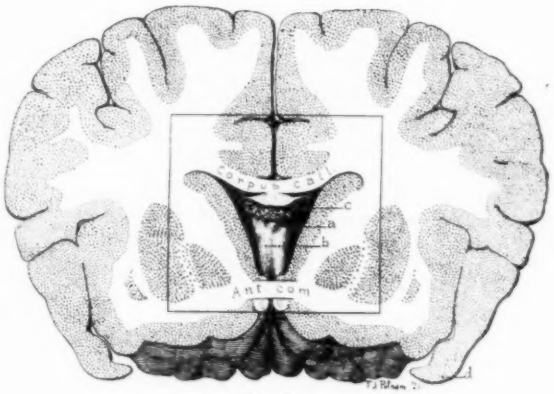

FIG. I.

FIG. II.

FIG. III.

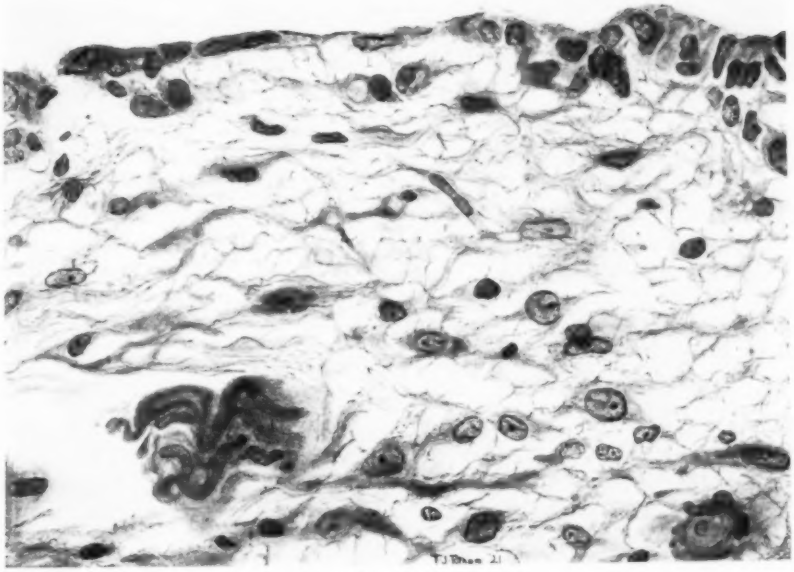

FIG. IV.

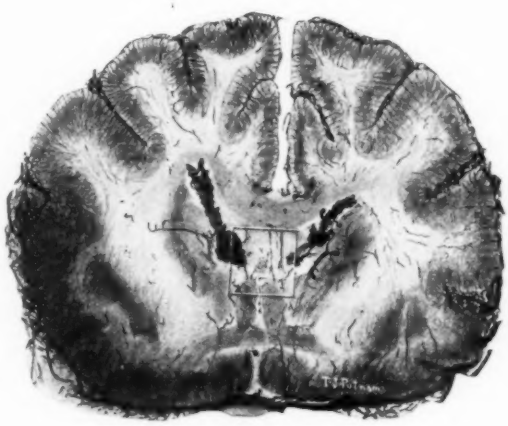

FIG. V.

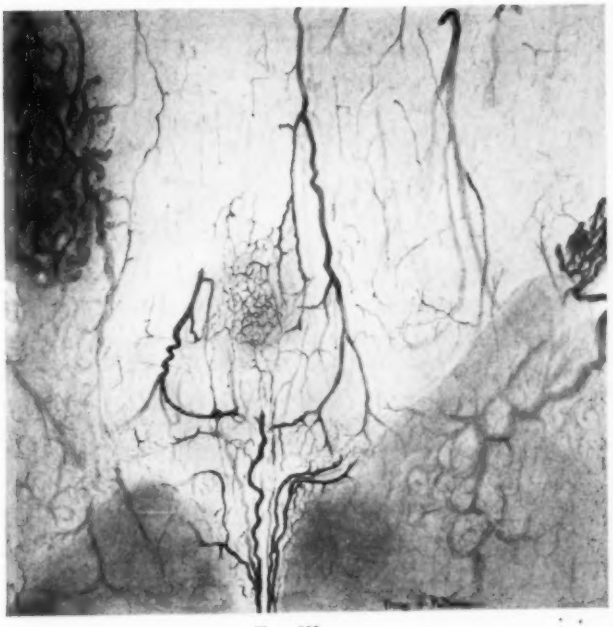

FIG. VI.

Fränkel's first case was that of a woman with a typical pseudomucinous cystadenoma of the ovary, which had ruptured. On the visceral peritoneum he found small implantation cysts, lined with epithelium a little lower than but otherwise similar to that in the ovarian tumor, and evidently actively producing the same pseudomucin.

The second case was that of a man who at autopsy was found to have a cystic appendix which had ruptured and from which had exuded gelatinous material. This material filled the pelvis and lower abdomen. Fränkel's report of this case marks the beginning of our knowledge of peritoneal pseudomyxoma in the male.

Pseudomyxoma Peritonei Ex Processu Vermiformi

Since Fränkel's case of pseudomyxomatosis of the peritoneum originating in the appendix, a number of other instances have been reported. Wilson, in 1912, mentions twelve cases and adds two more; since then a few additional reports have appeared, so that the occurrence of the condition is well established. Dodge, who collected from the literature a large number of cases of cystic appendix, found among them fourteen instances of pseudomyxoma peritonei caused by rupture or leaking of a pseudomucinous cyst of the appendix. In Seelig's recent article is a clear discussion of pseudomyxoma of the peritoneum in the male, which, he says, always arises from the appendix. On this point there is a difference of opinion, some holding that certain male cases are associated with a normal appendix. The actual origin in these cases is unexplained, but by some is assumed to be in the retroperitoneal tissues.

According to Seelig, epithelial cells in the exudate have been demonstrated; but the course of the disease does not seem to be dependent on the presence or absence of these cells. In his case the appendix was not seen, and presumably had been removed at an earlier operation. Cysts in the exudate were numerous, some lined with epithelial cells, some with mere shadows of cells. The spleen was very extensively invaded and the entire course was malignant. Lewis also shows that in her case there were many peritoneal cysts; and she further gives an excellent presentation of the chemistry of the condition.

Relative Importance Of Appendix And Ovary As Source.

Eden and others have described a very few instances, perhaps less than half a dozen, in which pseudomyxoma peritonei had undoubtedly originated from an ovarian cyst, but in which there co-existed an appendiceal cyst with gelatinous contents. In those cases in which the appendix and the ovary are both involved, it is not possible always to say offhand which is primary and responsible for the peritoneal condition.

Interesting suggestions have been made, pointing to a distinction between the peritoneal conditions caused by the appendix and the ovary, respectively. Fränkel in his careful postmortem studies of the peritoneum states that, when the masses of gelatinous material come from the ovary, they cause little reaction, and that at most they may be encapsulated by a very light membrane. He showed metastatic or implantation cysts, actively secreting. When the appendix is the source, he says, the peritoneum shows definite roughening, with organization of the foreign body and overgrowth by endothelium. Furthermore, no cystic metastases are found. Some authors however, with Seelig, state that epithelial cells may be extruded from the appendix also, and may at times multiply.

Fränkel assumed that the difference in the effect upon the peritoneum depends upon a chemical difference between the appendiceal and the ovarian secretions. Trotter says that in the appendix cases the jelly contains mucin, in the ovarian cases pseudomucin. In the literature, where the ovarian content has been studied at all, it seems to have been pseudomucin. As to the appendix cysts, in the large, almost complete series collected by Dodge, in only six cases was the chemical nature of the contents mentioned. In three cases the contents are reported as mucin, in two as pseudomucin and in one as colloid.

Certain it is that in the great majority of cases in which both the ovary and the appendix are the seat of pseudomyxomatous degeneration the ovary, and not the appendix, is responsible for the peritoneal involvement—as in the two cases reported below. Taking all cases of pseudomyxoma of the peritoneum in females, it is of course commonly found that the ovary itself is the only possible source. Pseudomucinous ovarian cystadenoma is relatively common. Wilson says that it forms from two to four per cent of all ovarian cysts. Appendiceal cysts, on the other hand, are relatively uncommon. Dodge, in 1916, collected 142 cases; I have found in the literature twelve others; and in the records of the Gynecological Department of The Johns Hopkins Hospital I have discovered nine more. From necropsy statistics Castle gives the incidence as 0.2 per cent, Corning as 0.5 per cent, Kelly and Hurdon as 0.42 per cent, Elbe as 0.5 per cent and Ribbert as a little over one per cent.

When appendiceal cysts do occur, however, they are most often of the pseudomucinous variety. Of the 142 cases reported by Dodge, in 81 the contents are described, and in 59 of these they were of a mucoid, colloid, gelatinous or pseudomucinous nature. In three of the nine cases found in the Hopkins gynecological records the appendix contained gelatinous material.

The evidence indicates, therefore, that in a given case of pseudomyxoma peritonei in a female the most likely source is in the ovary, especially if the peritoneal condition is extensive. The collection of gelatinous material from a cystic appendix usually is confined to the pelvis; although some cysts of the appendix form very large

tumors, and if ruptured might set free considerable amounts of secretion. Where a cystic ovary and a cystic appendix co-exist, the discovery of a point of rupture in one or the other organ will of course be decisive. When no point of rupture or perforation is evident, or when both ovary and appendix have been opened, a chemical examination of the exudate, as well as a histological examination of the peritoneum, may be helpful.

Two Cases Of Cystic Appendix Associated With Pseudomyxoma Peritonei Arising From An Ovarian Cyst

Case I.—*Gyn. Path. No. 23008 from the Church Home and Infirmary.* A white woman, 73 years of age, entered the hospital in April, 1917, complaining of marked abdominal enlargement. She had had the ordinary diseases of childhood, but since then had enjoyed good health until the present illness. She had married at 21 but had never been pregnant. The periods had always been irregular; the menopause had occurred at 45. The present illness had begun nine months before admission with loss of weight. For two months there had been slight vaginal bleeding, with remissions of a few days. Gastric symptoms had become marked only recently, with acid regurgitation and occasional vomiting. One month before admission the patient had noticed a lump in the lower right quadrant of the abdomen, and this swelling since then had increased rapidly in size.

Examination revealed a growth strongly suggestive of a cyst, evidently arising from the pelvis and partly filling the lower abdomen.

Operation by Dr. Cullen on April 25, 1917.—When the abdomen was opened, there was at once encountered a lumpy fluid reminding one of that found where colloid carcinoma exists. The cyst was multilocular in type and had very thin walls. It was removed with some difficulty. There was no evidence of metastasis. The appendix near its tip was one centimeter in diameter; it was removed.

Subsequent History.—Recovery from operation was uneventful. In December, 1917, the patient was reported well. By January, 1918, she had changed greatly; she had lost much flesh, was suffering from severe gastric symptoms, and the abdomen was full and tight. A little later she died.

Pathological Note (Dr. Wynne): The specimen consists of an appendix and an ovarian cyst. The appendix is 3.5 cm. long and 13.5 mm. in diameter. The surface is covered with adhesions. On section the lumen is greatly dilated, measuring one centimeter across. It contains a curious substance, which has the general appearance of a papillomatous mass embedded in gelatin. The ovarian cyst measures roughly 25 by 20 by 15 cm. It is subdivided by many thick partitions of grayish tissue. Many of the loculi are ruptured and a thick, yellow, gelatinous material exudes.

Microscopic Examination. Ovary.—The cyst walls have a compact, fairly vascular stroma, thrown up in irregular polypoid folds. The lining is of cylindrical epithelium with many goblet cells. The amorphous contents of the cyst stain with eosin. There are some hemorrhagic areas, with beginning organization. In some places the stroma is infiltrated with leucocytes, chiefly mononuclears.

Appendix.—The serosa is thickened and congested in places. No abnormality is seen in the muscularis. The mucosa is of very high cylindrical epithelium, most of the cells being of the goblet type. The mucosa is heaped up in papillomatous masses which nearly fill the lumen. The submucosa can be followed into the polypoid projections. No evidence of invasion or penetration of the muscle is seen.

Chemical.—The gelatinous material from the ovary, the appendix and the peritoneal cavity, when examined, had been in formalin, which may have affected its reactions. It could not be definitely identified as paramucin, pseudomucin or colloid. It was insoluble in alkali, acetic acid and mineral acid.

Case 2.—*Gyn. Path. No. 23580, Gyn. No. 23650.*—A married colored woman, aged 39 years, was admitted November 23, 1917, complaining of swelling of the abdomen and backache. The family history was unimportant. Menstruation had usually been regular, the last period having occurred in September, the preceding one in August. The last three periods had been very scanty. Prior to October there had been no missed periods except during pregnancy. No metrorrhagia had been noted. The patient had had four normal pregnancies, and no miscarriages. The present illness had begun four months before admission with epigastric pain at first only on exertion, later unassociated with it. For two or three months the abdomen had been becoming larger and increasing dyspnœa and orthopnœa had followed.

Examination showed a temperature of 97°, pulse 84, respirations 40. The woman was in great distress, vomiting, dyspnœic and absolutely orthopnœic. The abdomen was tremendously distended, but evidences of collateral circulation were only fairly distinct. A definite fluid wave was obtained. Pelvic examination showed simply bulging in the fornices and cul-de-sac. The lungs were clear except for signs of compression from below. The heart was somewhat dilated and there was a to and fro murmur, loudest at the tricuspid area. The blood pressure was moderately elevated, the vessels thickened. The extremities showed marked brawny œdema. The urine contained a trace of albumin and a few granular casts. The Wassermann reaction later proved negative.

Dr. Wynne decided that immediate relief for the dyspnœa was necessary, and under cocaine made a small incision below the umbilicus. The peritoneum was not recognized, and a cyst was cut into. There followed a gush of bloody fluid and gelatinous material, which was of a very clear light yellow color. This was let out gradually until about ten liters had been evacuated. The major operation was performed next day. A large amount of bloody fluid was found in the abdomen, with the same sort of gelatinous material evacuated the preceding day. This was removed and a cystic mass of considerable size brought up and found to have ruptured anteriorly. The walls were very thin and friable. This tumor had originated on the left side. A second ovarian cyst on the right side involved the broad ligament. The uterus was of normal size, with a few implantation nodules over it. In the cul-de-sac also were a number of implantations. The entire sigmoid, ascending and descending colon were covered by the gelatinous material, which was very adherent. The bowel walls were greatly thickened and œdematous. No nodules were seen in the omentum. The parietal peritoneum was thickened and rough, anteriorly about 4 mm. thick, very friable and in places marked by reddish excrescences. The appendix was swollen, larger at the tip than at the base. The uterus, adnexa and appendix were removed.

Subsequent History.—Recovery from the operation was smooth. On March 12, 1918, the patient seemed to be well except for symptoms of artificial menopause.

Pathological Note.—The right ovary, the tubes and the uterus are all in one mass. The uterus is normal. The right tube runs as a thick ridge over the top of the main mass, which represents the right ovary. This mass is irregular in shape, about 20 by 15 by 15 cm. It has a thick fibrous wall and many compartments, some of which have ruptured and collapsed. The contents are of a gelatinous nature. The walls of the locules are thin, hard and fibrous. Adherent to the inner walls are small masses, some of which are hemorrhagic or suggest vascularization. In a

few places are fine, whitish, papillomatous projections. No normal ovarian tissue is seen. The second part of the specimen represents the left ovary, and measures 15 by 10 by 8 cm. The gross picture of the tumor is essentially the same as that in the other ovary. The appendix is 3.5 cm. long, 1.75 cm. in diameter. It is ovoid in shape, feels cystic and has a definite constriction at the base. The surface is rough, opaque and a little injected. The walls are intact. On section the lumen is dilated to 8 or 9 mm. in diameter. The walls are firm, their thickness ranging from 2 to 6 mm. Near the base the lumen appears to be obliterated. Definite muscular layers can be distinguished and there seems to be an intact mucosa. No smaller cysts can be seen in the walls of the appendix. The lumen contains nothing but clear fluid.

Microscopic Examination. Ovaries.—The cyst walls consist of a dense stroma in heavy masses, compact and cellular. The epithelial lining is flattened, of cuboidal type, with a few goblet cells. There is a slight tendency to the formation of papillomatous projections.

Appendix.—The mucosa is flattened and there is but little infolding. In some transverse sections the mucosa is compressed, the muscularis normal, the lumen greatly distended. In other sections the submucosa is greatly thickened by inflammatory tissue and the lumen is much reduced. In places the submucous and muscular coats are infiltrated with many plasma cells and a few cells of the polymorphonuclear type.

Chemical.—The contents of the ovarian cysts and the abdominal jelly gave definite reactions for pseudomucin.

As to the apparent implantations in the peritoneum, unfortunately no specimens were taken at the time of operation.

SUMMARY AND COMMENT

In the male pseudomyxoma peritonei nearly always occurs from the rupture of a gelatinous cyst of the appendix. In rare cases it may possibly arise elsewhere, *e. g.*, in the retroperitoneal tissues.

In the female this condition may be due either to ovarian or to appendiceal disease. Sometimes both ovary and appendix are affected, and it may be difficult to say which is responsible for the peritoneal involvement. In such cases the chemical nature of the secretion may be valuable evidence.

In the first case here reported the jelly-like material of the abdomen obviously came from the ovary, as the appendix was unruptured. The appendiceal cyst is of the variety shown by Dodge's figures to be the most common, that is, it contains gelatinous material. It is, however, associated with a marked papillomatous condition of the mucosa.

The second case also is one of an ovarian cyst rupturing into the peritoneal cavity. The cyst of the appendix, a simple hydrops, is probably only coincident, and is a less frequent form of cyst than the first.

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HISTAMINE AS AN INFLAMMATORY AGENT

By WILLIAM BLOOM

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This research was undertaken with the view to determine whether histamine is positively chemotactic and whether it can cause inflammation. The author wishes to express his deep appreciation to Dr. Arnold Rich of the Department of Pathology for his sympathetic interest, aid, and advice in the carrying out of this research.

The process of inflammation has been studied in detail both histologically and *in vivo* so that the progression of the pathological process is known quite clearly; but we are still entirely ignorant of the substance, or substances,

that call forth this rather stereotyped reaction to injury on the part of the tissues. The beginning of the inflammatory process is the dilatation of the capillaries and smallest arterioles and venules in the affected area; this is followed ordinarily by an exudation of fluid from the widened vessels and the migration of leucocytes into the inflamed area. Inflammation proceeds in fundamentally the same manner whether it be provoked by bacteria or by necrosis of cells in the absence of bacteria; so that one feels that perhaps the inflammatory inciting substance or substances may be something liberated from damaged tissue cells rather than extraneous bacterial toxins. Indeed, Massart and Bordet [1] have shown that dead tissue extracts are positively chemotactic, although the actual chemotactic substance has not as yet been isolated. The substance or combination of substances that causes inflammation must satisfy the following demands: (a) it must be a capillary poison, (b) it must be present in all tissues, (c) it must be liberated through trauma and bacterial action on proteins, and (d) it must attract leucocytes to the focus of injury.

The work of Dale, Laidlaw and Richards [2] has directed considerable attention to histamine (B—iminazolylethylamine) because of the dramatic effects upon the circulation which minute amounts of the substance produce, and because of the striking similarity between surgical shock and the condition induced by the intravenous injection of histamine. Dale and his co-workers reached the conclusion, through indirect methods, that the effects of histamine on the circulation are the results of its action as a capillary poison—causing dilatation and increased permeability of the capillaries. The capillary dilator effect of histamine was not observed directly by these workers; but recently Hooker,[3] studying the skin capillaries *in vivo*, has watched capillary dilatation occur under the influence of histamine, and Rich [4] has demonstrated histologically that the capillaries of the omentum (of the cat), and the smallest arterioles and venules as well, are markedly dilated, and that previously closed capillaries are opened up during both histamine shock and local applications of histamine. Abel and his co-workers [5] have shown that histamine is a constituent of all animal tissues. The work of Bayliss [6] and Cannon [7] indicates that crushing the leg muscles of a cat will produce in the animal a condition that resembles histamine shock. Ackerman [8] and Mellanby and Twort [9] have shown that histamine is formed by the action of bacteria on histidine, an amino acid constituent of all proteins. In view of these results it was thought that, if it could be shown that histamine was positively chemotactic, a possible explanation of inflammation would result.

It was realized in approaching this problem that some means would have to be found to localize the histamine in the tissues; since many highly inflammatory substances produce no reaction when injected intravenously (Adami).[10] It is because of this that it was thought that the failure of Paul [11] to produce leucocytosis by intravenous injection of histamine in the rabbit was inconclusive.

Methods

Because of its susceptibility to histamine the cat was used in the following experiments. Hoffman, La Roche histamine dihydrochloride was used in all experiments.

I. A large number of cats were injected intraperitoneally with 3 mg. of histamine in 10 c.c. of physiological saline and an equal number of cats were injected with saline alone as controls. The animals were killed with ether at ten, fifteen, thirty minutes, and one, two, four, six and eighteen hours after injection. Under aseptic conditions the peritoneal cavity in these animals was opened and inspected for fluid. When any fluid was present, it was collected and tested for free cells and for its ability to coagulate, and cultured to exclude the possibility of bacterial contamination. These experiments were performed to determine whether, if histamine be a capillary poison, there would be a difference in the transudate caused by it and by saline.

II. To study the more locally inflammatory action of histamine, the following subcutaneous injections in the ear were made. Sufficient histamine, 1:100, in physiological saline, was injected to raise a bleb 1 cm. in diameter. Control injections were made with physiological saline alone. The blebs would disappear in one or two hours. Forty, eighty, one hundred and ten minutes and twenty hours after the injections had been made the cats were killed with ether, the ear removed, fixed in a formaldehyde-Zenker fluid and then sectioned after imbedding in paraffin. The sections were stained with hæmatoxylin and eosin and with MacCallum's bacterial stain. In order to localize the histamine for longer periods of time in the subcutaneous tissues, 20 mg. of histamine-hydrochloride crystals suspended in 0.5 c.c. of olive oil were injected subcutaneously in the ear. Control injections were made with croton oil, 1:200 in olive oil. In these experiments the same technique for preparing the sections was employed as that used for the saline injections. Intra-muscular injections of paraffin oil and of paraffin oil containing 20 mg. of histamine crystals in suspension were made.

III. It was thought desirable, however, to keep the histamine localized for longer periods of time than the previous methods permitted. Accordingly, collodion capsules containing histamine 1:100 in physiological saline and control capsules containing physiological saline alone were placed, with as little injury to the tissues as possible, between the external and internal oblique abdominal muscles of the cat. Other control experiments were made with capsules containing 5% lactic acid, a negatively chemotactic substance, and with capsules containing croton oil, a highly inflammatory substance. The capsules

were allowed to remain in the animals for periods of time varying from eighteen to twenty-two hours and after the animals had been killed with ether the capsules were removed with the contiguous muscle and treated histologically as in I.

The collodion capsules were made by drawing a viscous solution of collodion into glass tubes of 4 mm. bore; the excess collodion was allowed to drain out, and the thin-walled, hollow, collodion cylinders were extracted from the glass moulds with the aid of cold water, after the ether-alcohol mixture had evaporated. The tubes were then filled with the substances to be experimented with and were tied off at the desired lengths with silk thread. To prevent the collapse of the capsules during autoclaving, it was found necessary to keep them immersed in the same liquids as those that they contained. To test the ability of histamine to dialyze through collodion, capsules containing histamine 1:100 in physiological saline were suspended in physiological saline and after twenty-four hours the dialysate on being injected into a cat produced a typical histamine fall of blood pressure.

IV. To test the purely chemotactic properties of histamine *in vivo*, capillary glass tubes containing histamine 1:100 in physiological saline and control tubes containing physiological saline were buried subcutaneously in the cat. The tubes were sealed at one end to prevent any capillary attraction from drawing in those leucocytes which would respond to the injury produced in planting the tubes. After twenty hours the tubes were removed from the animals and inspected with a microscope to see whether any leucocytes had wandered into the bore—as they should do if histamine is positively chemotactic.

V. Tubes similar to those used in the preceding experiments were surrounded by leucocytes on a warm microscope stage to determine the chemotactic properties of histamine *in vitro*.

RESULTS

I. There was practically no difference between the effects produced by intraperitoneal injections of histamine—3 mg. in 10 c.c. of physiological saline—and of physiological saline alone. The transudate, when present, consisted of a few free cells and a fluid capable of clotting. The greatest amount of fluid was obtained from the peritoneal cavities of those animals killed within one hour after injection. Animals killed after longer intervals than one hour showed very little and those killed at eighteen and twenty hours showed no fluid whatever. It was interesting to note, however, that in those animals which were killed one half hour or less after injection, there was a pink flush in the intestines of only those which had been treated with histamine.

II. Subcutaneous injections of histamine, 1:100 in physiological saline, caused a slight inflammatory reaction that neither qualitatively nor quantitatively differed from the inflammation caused by subcutaneous injection of physiological saline.

Subcutaneous injections of olive oil and of olive oil with histamine crystals suspension caused slight inflammatory reactions. In no case was there a difference between the oil or the oil with histamine, while subcutaneous injection of croton oil, 1:200 in olive oil, caused an exceedingly intense inflammation at the side of injection, very much more violent than that caused by the oil or oil and histamine.

Intramuscular injections of paraffin oil and paraffin oil with histamine crystals in suspension gave no results. The paraffin oil was not absorbed and probably did not permit the histamine to diffuse into the contiguous tissues. In no case was any inflammatory reaction whatever detectable, although the presence of the oil in the muscles was quite obvious.

III. The collodion capsules containing histamine 1:100 in physiological saline called forth marked inflammatory reactions consisting of fibrin, very many polymorphonuclear neutrophilic leucocytes and a few lymphocytes. The inflammatory reaction is most marked around the capsules, which it surrounds entirely, but it can also be traced along the line of separation of the muscles, that is, where the muscles had been separated so that the capsules might be planted. But in no case was the reaction in any way different from that caused by the collodion capsules that contained only physiological saline. Collodion capsules containing 5 per cent lactic acid called forth a very slight reaction, shown by the presence of a few leucocytes and a little fibrin—a picture that contrasted quite strongly with that produced by collodion capsules containing croton oil; in the latter case a violent inflammation was caused. All of the muscles surrounding the capsules were heavily infiltrated with leucocytes; there was most intense reaction around the capsule and all of the blood vessels in the section were greatly distended with reds and leucocytes.

IV. Glass tubes containing histamine do not attract leucocytes *in vivo*.

V. Leucocytes do not wander into glass tubes containing histamine in physiological saline *in vitro*.

CONCLUSIONS

1. A number of experiments were performed to determine the inflammatory inciting and chemotactic properties of histamine.

2. In none of these experiments did histamine produce an inflammatory reaction more marked than that caused by the control experiments.

3. Histamine is indifferent with respect to chemotaxis *in vivo* and *in vitro*.

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TREATMENT OF NON-ENCAPSULATED BRAIN TUMORS BY EXTENSIVE RESECTION OF CONTIGUOUS BRAIN TISSUE*

By Walter E. Dandy

Gliomata comprise probably 80 per cent of brain tumors; they are never encapsulated, some are circumscribed, others infiltrating. Until just lately, the fact that they invariably recurred has rendered operative procedures apparently hopeless. In recent work, however, we have made trial of careful resection, *en masse*, of a zone of normal brain tissue with the tumor in the center. We have removed an entire right or left frontal lobe without any observable mental or other after-effect. Whole right temporal or right occipital lobes have been removed with only a contra-lateral homonomous hemianopsia. In two instances, it has been necessary to resect the right temporal and the occipital lobe. Practically complete resection of one or two lobes of the cerebral hemispheres has been done in about 10 instances, but partial resection of a lobe with the tumor is frequently all that is necessary. The exact amount of brain tissue which must be removed depends on the position, size and character of the tumor. Partial or even complete resection of lobes of the brain entails very little operative risk. The two largest resections were done when the patients were unconscious, and complete recovery followed.

Similarly, gliomata of the cerebellum can be removed by resecting large portions of the cerebellum. Three times we have removed the entire vermis and no symptoms have followed. Two boys, 10 years old, are well and working, four years after removal of the tumor together with all of the vermis and about half of each lateral lobe of the cerebellum. What parts and how much of the cerebellar lobes can be removed without symptoms, I am not prepared to say. Resections of the cerebellum are more serious than those of the cerebrum, but in adults there has been a very low mortality.

Whether gliomata of the cerebrum or cerebellum can be permanently cured by this method, time alone must determine. Several of our patients are living and well 3 and 4 years without signs of recurrence.

One cannot always tell from the surface appearance of the tumor, whether a complete resection will be possible. It has never seemed advisable to resect the leg center of the pre-Rolandic area, but in several cases the face area and occasionally the arm center has been removed. Three times it has been necessary to remove Broca's area, apparently completely and to a depth of 2 to 3 cm. Although a complete motor aphasia appeared at once, the speech began to come back in a week or 10 days and gradually returned to normal. In two cases the left occipital lobe has been resected, but not far enough forward to produce visual aphasia. It has never seemed justifiable to attempt to cure a patient of a tumor by resection of the left temporal lobe. In several cases the various ventricles of the brain have been resected. If the dura and scalp are closed very carefully, the open ventricle will do no harm. When tumors in the cerebral hemispheres are localized before paralyses develop, they can be removed without causing paralyses, and if paralyses are present at the time of operation, these will usually become less after the tumor has been removed. The value of early localization of the growth is self-evident.

The removal of these large areas of brain tissue is made possible by the application of Dr. Halsted's well-known principles of careful handling of tissues, and painstaking hæmostasis, so that loss of blood is prevented as far as possible.

II. DIAGNOSIS, LOCALIZATION AND REMOVAL OF TUMORS OF THE THIRD VENTRICLE*

By Walter E. Dandy

In one patient, a woman, 24 years of age, the only symptoms were those referable to intracranial pressure. By cerebral pneumography, it was determined that each lateral ventricle was greatly enlarged, but there was no communication between them. Hence we concluded that there must be a tumor in the third ventricle and occluding each foramen of Monro; that the tumor must be small, because neither ventricle was dislocated away from it. A large bone flap was turned down as in a pineal

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approach, and the corpus callosum split posteriorly for about 5 to 6 cm. The right lateral ventricle was then opened through the mesial wall at the septum lucidum. No tumor could be seen; the right foramen of Monro seemed normal on inspection from this lateral ventricle, but a probe would not pass through it, an obstruction being encountered in the third ventricle. The foramen of Monro was then widened and a small encapsulated, spherical tumor, about 1 cm. in diameter, easily shelled out *in toto*. The tumor was of ependymal origin. The patient recovered. Without cerebral pneumography localization of the tumor and consequently its removal would have been impossible.

III. CEREBRAL VENTRICULOSCOPY*

By WALTER E. DANDY

On two occasions, it has seemed advisable to inspect a lateral ventricle. This was done in one instance through a small cystoscope and in a second an attempt was made with the help of a small operating ventriculoscope to remove and fulgurate the choroid plexus. Both operative attempts were only partially successful, because the instruments, in their relatively primitive construction, were not quite adaptable, and it therefore became necessary to remove the plexus in the usual way. The inspection of the ventricles was all that could have been desired. It was possible to see practically the entire extent of the lateral ventricle, the foramen of Monro, the septum lucidum with numerous perforations in it, and the entire extent of the choroid plexus including the glomus. The blood vessels in the wall of the ventricle were easily visible. In one instance, we could distinguish the opening of a defect in the wall of the ventricle—the mouth of an encephalocele. The indirect means of determining the intracranial contents are now so satisfactory that ventriculoscopy will be indicated only occasionally. There may, however, at times be cases in which a direct inspection will be useful. How useful the operating ventriculoscope will be, can scarcely be predicted. I have used both air and water as media in the ventricles; the former, which can be easily introduced, is by far the better. For inspection alone, the instrument introduced into the ventricle need be little larger than the ordinary ventricular needle.

IV. AN OPERATION FOR THE REMOVAL OF LARGE PITUITARY TUMORS*

By WALTER E. DANDY

This procedure is an extension of Heuer's operation, which is by far the most practical and useful of all yet suggested. In many cases, when the tumor is small or when the lesion is a simple cyst, Heuer's approach is adequate, but most pituitary tumors are very large when they come for operation, so that total removal is possible only with much more room. The exposure of the tumor is enlarged 4 or 5 times by section of one optic nerve. The nerve supplying the eye in which the vision is worse, or in which the vision has been first lost, is chosen for section. If the difference is slight, the right eye is always selected.** This supplementary procedure renders it possible to retract the brain to such an extent that almost any tumor of the pituitary can be completely removed. Adenomas of the pituitary (the most frequent hypophyseal tumors) are first resected intracapsularly, the loose capsule is then pulled forward in stages and eventually removed flush with the sella. This method I have used for the past four years. Up to the present time we have not had a recurrence, two patients having gone 4 years. The vision in the intact eye returns, the exact extent of recovery depending on the duration of blindness before operation. Complete removal of these tumors is, of course, a serious operation, the danger, in a general way, being in proportion to the size and character of the tumor and the strength of its capsule.

V. AN OPERATIVE PROCEDURE FOR HYDROCEPHALUS*

By WALTER E. DANDY

Strictures of the aqueduct of Sylvius recur after any attempt to restore the lumen. For this reason, if treatment is to be successful, the fluid must be sidetracked into its normal channels. With this in mind, a procedure which apparently is anatomically correct has been devised, to supersede any direct attack on the aqueduct. This consists in removing the floor of the third ventricle. A small opening is made in the skull and dura in the frontal region, the frontal lobe is elevated until the

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** These patients are nearly always blind in one or both eyes at the time of operation.

bulging third ventricle is well exposed. Usually, it is necessary to divide one of the optic nerves, for in hydrocephalus these nerves are very short; usually the chiasm lies directly on the anterior border of the sella turcica. This opening in the floor of the third ventricle affords an exit from the dilated ventricles, so that the fluid can now pass directly into the cisterna chiasmatis and interpeduncularis—the normal distributing centers for cerebrospinal fluid. We now have proof that an opening made in the fourth ventricle will remain permanently patent, and this encourages the hope and belief that this opening in the floor of the third ventricle will also be permanent. The ventricular wall is a very thin membrane and offers a minimum of glia tissue to repair the defect.

This procedure is by no means analogous to making an opening in the roof of the third ventricle. The latter can have no beneficial result because the fluid escapes into the subdural space where the absorption is slightly, if at all, greater than in the ventricles. Moreover, the opening is through cerebral tissue which proliferates and closes it, unless a good deal of the brain has been destroyed.

We have employed this method 6 times. No claim is made for its success. Time alone will decide. If successful, it should also be applicable to occlusions of the foramina of Luschka and Magendie and to those cases of communicating hydrocephalus in which the cisterna chiasmatis and interpeduncularis are patent.

VI. DIAGNOSIS AND LOCALIZATION OF SPINAL CORD TUMORS*

By Walter E. Dandy

In 1919, a report of intraspinous injections of air was presented. In several cases, the air was found in skiagrams of the head and proved to be useful in defining blocks in the subarachnoid space, resulting from tumors or inflammations. The air was shown in the upper part of the spinal canal and it was suggested that blocks of the spinal canal should be similarly demonstrable or could be excluded when not present. In five cases of possible spinal cord tumors we have made intraspinous injections of air. In four, it passed through into the cranial chamber and was demonstrable in the subarachnoid space and the ventricles, so that tumors could be excluded, and other diagnoses could be considered.

In the case of one of Dr. H. Thomas's patients the upper margin of the air was shown at the fifth cervical vertebra; sharp pains were at once carried down the sixth cervical nerves on each side (the site of the tumor). No headache occurred in this case, because the air did not reach the cranial chamber. The skiagram of the head also failed to demonstrate air either in the cerebral sulci or in the ventricles. As the Wassermann for the blood and spinal fluid was positive, antisyphilitic treatment was instituted with the result that the patient recovered completely from the paralysis. Before his discharge from the hospital, the intraspinous air injection was repeated; headache at once appeared; and air was shown in the skiagram of the cerebral ventricles. No pains developed along the cranial nerves at this time. It was evident that the tumor had been absorbed, and that air could pass freely through the region which previously had been blocked. This method should give the same results as the combined spinal and cisternal punctures of Ayer. It is hoped that the air shadow will be sufficiently clear not only to permit the diagnosis of a subarachnoid block but also to establish its localization. How sharply shadows in the thorax and abdomen will be defined, I do not know.

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SPONTANEOUS LABOR OCCURRING THROUGH AN OBLIQUELY CONTRACTED, KYPHOTIC, FUNNEL PELVIS*

By J. Whitridge Williams.

This case seems worthy of being recorded for two reasons—first, because it affords a striking illustration of the mechanism by which certain skeletal changes may affect the shape of the pelvis, and secondly, because the conservatism with which the labor was conducted afforded conclusive evidence of the ability of the uterine cicatrix following Caesarean section to withstand the strain of labor, as well as to demonstrate the fallacy of the dictum "once a Caesarean, always a Caesarean."

The patient was a twenty-one year old colored girl, who was markedly deformed by kyphotic changes in the vertebral column and by ankylosis of the left hip and knee originating from tuberculosis developing during the second year of life. The two previous pregnancies had been ended by Caesarean section in another city in 1919 and 1920 respectively, and she was admitted to the ward on September 26, 1921, under the belief that she was in

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labor at term—the last menstrual period having begun on December 20, 1920.

In view of the previous history, the woman entered the service for a third Caesarean section and, as she was anxious to avoid the possibility of its repetition, it was tentatively proposed to remove the uterus by supra-vaginal amputation after evacuating its contents. On examination, there were occasional uterine contractions, and a small child presented in R. O. P. with its head deeply engaged in the pelvic cavity. In the absence of disproportion, I declined to do a radical operation and predicted a spontaneous outcome, but advised that forceps be applied early in the second stage in order to prevent unnecessary strain upon the scars of the previous sections.

As it proved that the patient was not in labor, I examined her thoroughly the next day with the following results: a frail young woman weighing 83½ pounds and measuring 130 cm. was lying on her back in bed. The heart and lungs were normal. The thorax was unusually arched and, with the large sagging breasts and pendulous abdomen, presented a peculiar picture. The fundus of the uterus reached to within three fingers of the xiphoid, and the abdominal enlargement projected in such a way that its lower part extended vertically downward. A jagged Caesarean section scar extended one-third above and two-thirds below the umbilicus. A small child lay in R. O. P. with the head deeply engaged. In the left groin just below Poupart's ligament was an old scar indicating the point of discharge of a psoas abscess. The left leg was atrophic as compared with the right, and was fixed at the hip-joint in slight flexion. The knee was flexed and completely ankylosed, while a long indrawn scar upon its posterior surface indicated the site of an operation which had been performed when the patient was five years of age.

Upon inspecting the standing patient from the front, (Fig.1) one was impressed by the extreme shortness of the torso, the pendulous condition of the abdomen, and the fact that the body weight was supported entirely by the right leg, while the toes of the left foot scarcely touched the floor. The abdomen was so pendulous that no trace of the abdominal cicatrix was visible, and the umbilicus lay 7 cm. below a line joining the iliac crests. When viewed from the rear, four striking features were noted. First, the presence of a double kyphosis, with one gibbus in the upper dorsal and the other in the lower lumbar region; second, tilting of the entire vertebral column so that the right shoulder was higher than the left; third, collapse of the torso to such an extent that the lower-most ribs were in contact with the iliac crests, which was indicated externally by a deep transverse furrow of the skin on either side; and fourth, oblique tilting of the pelvis so that the right iliac crest was 2 cm. higher than the left.

As is shown by Figure 2, there is nothing remarkable about the upper gibbus, but the lower one, which is separated from the former by a moderate lordosis, is of interest, partly on account of its location, but particularly because its lower limb passes directly into the sacrum. The existence since childhood of such a lumbar kyphosis led us to suppose that the pelvis would be funnel-shaped, while the unilateral lameness would give rise to an oblique contraction. Careful examination confirmed both suppositions.

External pelvimetry gave the following measurements: 21, 23, 27 and 17.5 cm., and showed that the pubic arch was asymmetrical and somewhat narrowed, with the transverse and anteroposterior diameters of the outlet measuring 7.25 and 10.75 cm., respectively. On internal pelvimetry, the diagonal conjugate was found to measure 12 cm. and the entire right side of the pelvis was flattened and pushed upward, inward and backward, while the left side presented normal curvatures. At first glance it appeared that we had to deal with a typical generally contracted funnel pelvis, associated with oblique contraction resulting from the unilateral lameness. Closer consideration showed that this was not the case, as the diagonal conjugate of 12 cm., although slightly shorter than normal, was disproportionately large for such a pelvis. On the contrary, we were forced to conclude that it was really relatively lengthened, while both the transverse and anteroposterior diameters of the inferior strait were absolutely shortened. In other words, all of the criteria for a kyphotic funnel pelvis were fulfilled.

Figure 3, which represents an X-ray of the superior strait, clearly shows the existence of the oblique contraction and demonstrates that it had resulted from the unilateral lameness. In this instance, the patient since early childhood had supported her body weight almost exclusively upon her right leg, with the result that with each step a greater upward and inward force had been exerted through the right than through the left acetabulum, and consequently the right side of the pelvis had become flattened and slowly forced upward, inward and backward, while the left side had developed normally, thus giving rise to an oblique contraction involving the entire pelvis, but more particularly the superior strait, which has assumed an obliquely ovate form. That the contraction was not more pronounced is probably attributable to the fact that the patient has always walked with a crutch, and thereby diminished to some extent the extreme mechanical possibilities of her lameness.

Reverting to the kyphosis. The presence of a "hump back," no matter what the situation of the gibbus, is always a source of anxiety to a pregnant woman and causes her to anticipate serious difficulty at the time of labor. Experience, however, shows that such fears are generally exaggerated, as the great majority of kyphotic deformities do not lead to serious dystocia. This is due

to the fact that when the gibbus is situated in the cervical or dorsal region, or even when it is dorso-lumbal in character, the development of a marked lordosis below it results in such compensation, that the body weight is transmitted to the base of the sacrum in such a manner that the pelvis is not affected. On the other hand, as has been shown by the researches of Breisky, Chantreuil, Tarnier and others, when the kyphosis involves the lumbar region, there is no longer any possibility for compensation, as the lower limb of the gibbus articulates directly with the base of the sacrum, with the result that the body weight is transmitted to the upper end of the lower limb of the gibbus in such a way that a parallelogram of forces is developed which resolves itself into two factors—one directed downward and the other downward and backward. The latter tends to displace the lower limb downward, and at the same time to draw it backward. As its lowermost extremity is firmly united to the base of the sacrum, this results in a rotation of the sacrum about its transverse axis so that its base becomes retropulsed, while its tip is displaced forwards, thereby increasing the length of the conjugata vera and diminishing that of the antero-posterior diameter of the inferior strait. At the same time the sacro-iliac joints are spread apart, with the result that the innominate bones rotate about their transverse axes, so that the ischial spines and the tubera ischii approach one another and thus diminish the transverse diameters of the plane of least pelvic dimensions and of the inferior strait with the production of a funnel pelvis, as is demonstrated by the example before us.

In most kyphotic funnel pelves which have been studied in the dry state a further change has been noted—namely, a lengthening of the sacrum from tip to base, as well as a slight diminution in its concavity arising from the fact that the traction exerted upon its base tends to draw the bodies of the sacral vertebrae out beyond the level of the alae. Whether this was present in the pelvis under discussion I am unable to state, but no evidence of it was apparent upon palpating the sacrum or in the X-ray plate.

The patient fell into labor early in the morning of October 13, 1921, and three hours and twenty minutes later vaginal examination showed that the cervix was fully dilated and the head on the pelvic floor in R. O. A. The membranes ruptured spontaneously at this time, and low forceps were applied in order to spare the uterine cicatrix the strain incident to expulsion, and a male child weighing 2420 grams and measuring 46 cm. in length, with a biparietal diameter of 9 cm., was easily delivered without injury to the perineum.

The convalescence was uneventful except for a mild colon bacillus pyelitis, which developed three days before the onset of labor and caused a febrile reaction, which continued until the fifth day of the puerperium. The patient was discharged in excellent condition on the 15th day and suckled her child, which on discharge exceeded its birth weight by 80 grams.

The labor was of interest from two points of view. First, that the small child passed through the abnormal pelvis without difficulty, and secondly, that neither the distention of the uterus incident to pregnancy nor the strain of the second stage of labor had any untoward effect upon the scars of the previous sections.

Through the courtesy of the hospital at which the previous Caesarean sections had been done, we learned that the children weighed 2880 and 2490 grams, respectively, as compared with 2420 grams in the present instance. This at once raises the question as to whether radical operative interference was necessary. While criticism is not permissible unless one is in possession of all the facts, it appears probable that the second labor, at least, would in all probability have ended spontaneously had it been given a chance, and that the actual indication for the second Caesarean section was fear of the behavior of the uterine scar following the first operation. The result in our hands shows that such fears were groundless, and brings us to the discussion of the behavior of the Caesarean scar in general.

A priori, one would be inclined to believe that the scar tissue in the cicatrix would represent a *locus minoris resistentiae,* which might predispose to rupture in subsequent pregnancies, and the actual occurrence of such accidents indicates that the danger is a real one. I shall not, however, discuss the question at any length, as an extensive article by Dr. Thomas O. Gamble based upon the experience of our service has just been published.

In Gamble's paper, evidence was adduced to prove that, while rupture sometimes occurs, its frequency is much less than is currently taught. This is due to the fact, which we first demonstrated, that when the uterine wound has been properly sutured, and infection has not occurred, microscopic examination shows a surprising lack of scar tissue, and demonstrates that muscular fibres cross the site of the old incision in all directions just as if it had never been made. Indeed, in a number of uteri which were removed at a second or third section, no trace of the previous incision could be discovered, except perhaps a slight furrow upon the external and internal surfaces of the anterior uterine wall, and in certain specimens even this was lacking.

On the other hand, if the incision has not been properly sutured, and more particularly if infection has occurred, good union may not obtain, with the result that the uterine wall is thinner at the site of the old incision than elsewhere. In such circumstances, it is conceivable that, as the distension of the uterus increases, the thinning may become so accentuated as to lead to rupture with its serious consequences.

FIG. 1.—Showing shortened torso, pendulous abdomen and body weight borne upon right leg.

FIG. 2.—Showing double gibbus and collapse of torso.

FIG. 3.—Showing obliquely ovate superior strait.